SEM STUDY OF THREE DIFFERENT INTERFACES BETWEEN SEALER AND ROOT END FILLING MATERIALS – AN IN VITRO STUDY

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in partial fulfillment of the requirements

for the degree of

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BRANCH – IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600 032

2014 – 2017

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I hereby declare that this dissertation titled **"SEM STUDY OF THREE DIFFERENT INTERFACES BETWEEN SEALER AND ROOT END FILLING MATERIALS – AN IN VITRO STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.P.Hemalatha, M.D.S., Professor, Head of the Department,** Department of Conservative Dentistry and Endodontics, Best Dental Science College, Madurai – 625104.

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DECLARATION

TITLE OF DISSERTATION	SEM STUDY OF THREE DIFFERENT INTERFACES BETWEEN SEALER AND ROOT END FILLING MATERIALS – AN IN VITRO STUDY
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DURATION OF THE COURSE	3 YEARS
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This agreement herein after the "Agreement" is entered into on this day 29th Dec 2016, between the Best Dental Science College represented by its **Principal** having address at Best Dental Science College, Madurai – 625104, (hereafter referred to as, 'the College')

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And

Mr. Dr.C.A.ANAND YOKESH aged 37 years currently studying as **Post Graduate student** in Department of Conservative Dentistry & Endodontics, Best Dental College, Madurai- 625104 (herein after referred to as the 'PG/Research student and co-investigator')

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(Dr. C.A.ANAND YOKESH)

LIST OF ABBREVATION USED

(IN ALPHABETICAL ORDER)

ABBREVATION	WORD EXPLANATION
CPC	Calcium Phosphate Cement
EBA	Ethoxy Benzoic Acid
E Faecalis	*Enterococcus Faecalis*
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme Linked Immunosorbent assay
Er:YAG	Erbium:Yttrium Aluminium Garnet
FTIR	Fourier Transform Infrared Spectroscopy
GIC	Glass Ionomer Cement
GMTA	Gray Mineral Trioxide Aggregate

HAC	Hydroxyapatite Cement
IRM	Intermediate Restorative Material
MTA	Mineral Trioxide Aggregate
NRFM	Novel Root End Filling Material
OMTA	Orthograde Mineral Trioxide Aggregate
p Value	Probability Value
PC	Portland Cement
PBS	Phosphate Buffered Saline
RMTA	Retrograde Mineral Trioxide Aggregate
SD	Standard Deviation
SEM	Scanning Electron Microscopy

SPSS	Statistical Package for Social Sciences
STF	Synthetic Tissue Fluid
US	Ultrasonics
WMTA	White Mineral Trioxide Aggregate
XRD	X-Ray Energy Dispersive Analysis

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The success of endodontic treatment depends mainly on perfect root canal preparation and adequate endodontic seal thereby preventing microorganisms and their products from reaching the apical and periapical tissues[1]. Any pathology which involves only the pulp can be successfully treated by conventional non surgical root canal treatment with a success rate of 96%. But when pathology extends into the periradicular tissues, chances of success are comparatively low. Such cases may require surgical intervention for establishing complete debridement and hermetic seal between the intraradicular and extraradicular tissues[2].

The aim of periradicular surgery is to remove the etiology of the disease and provide a favourable environment for surgical wound healing. The surgical procedure involves 3mm resection of the root, since it is the area where 98% of apical ramifications, lateral and accessory canals are present. It is followed by root end preparation and restoration with root end filling material. The purpose of endodontic surgery is the apparent elimination of the persistent microorganisms in the apical third, depriving them of nutrient supply and providing adequate apical seal[3]. The main indications for periradicular surgery are failed non surgical endodontic treatment, need for surgical drainage of periodontal or periapical abcess, calcific metamorphosis of the pulp space, procedural errors, anatomic variations, biopsy, corrective surgery and replacement surgery[4].

According to the Washington study, Ingle et al[2] reported that over two thirds of endodontic failures were related to incomplete cleaning and obturation of root canals. Harty et al[5] have also reported that the majority of non-surgical endodontic procedures which fail do so was due to inadequate apical seal. Solubility of sealer in periradicular tissue fluids may result in delayed leakage and long-term failure if a root-end filling is not placed[6]. Because most endodontic failures occur as a result of leakage of irritants from pathologically involved root canals, the root-end filling material should provide an apical seal to an otherwise unobturated root canal or improve the seal of existing root canal filling materials and be biocompatible with periradicular tissues[7]. The purpose of root end filling is to establish a seal between the pulp canal space and the periapical tissues. The quality of apical seal provided by the root end filling material during periradicular surgery is considered critical for a successful outcome[8].

An ideal root end filling material should able to seal the contents of the root canal system within the canal and prevent egress of any bacteria, bacterial byproducts, or toxic material into the surrounding periradicular tissues[9]. According to Gartner and Dorn , Kim et al., Chong , root end filling materials should adhere or bond to tooth tissue and seal the root end three dimensionally, not promote and preferably inhibit the growth of pathogenic microorganisms, be dimensionally stable and unaffected by moisture. It should also be well tolerated by periradicular tissues with no inflammatory reactions, stimulate the regeneration of normal periodontium, be nontoxic both locally and systemically, not corrode or be electrochemically active, not stain the tooth or the periradicular tissues, be easily distinguishable on radiographs, have a long shelf life and be easy to handle[10].

Various root end filling materials have been used in the past such as Amalgam, Glass ionomer cement, Intermediate restorative material, Super EBA, Gold foil, Cavit, Zinc oxide Eugenol and so on[11]. Recently newer materials like MTA, Biodentine have been introduced and they seem to provide better apical seal[7].

Mineral Trioxide Aggregate (MTA) is a calcium silicate based endodontic material which was introduced by Mahmoud Torabinejad at Loma Linda University, California, USA in 1993. MTA is derived from Portland cement that is composed mainly of tricalcium silicate and dicalcium silicate. Studies on MTA reveal that it exhibits good sealing ability, excellent long term prognosis, relative ease of manipulation, good biocompatibility and favors tissue regeneration as well[12]. Thus, it is currently considered as the most promising material for root-end filling (Chong et al.2003, Saunders 2008, Baek et al. 2010), direct pulp capping (Nair et al. 2008, Okiji & Yoshiba 2009, Mente et al. 2010), perforation repair (Main et al. 2004) and apical barrier for teeth with necrotic pulps and open apices (Simon et al. 2007)[13].

Several new calcium silicate based materials have recently been developed (Asgary et al. 2008, Camilleri 2008, Gandolfi et al. 2008, Gomes-Filho et al. 2009), aiming to overcome some of the drawbacks of MTA, such as its handling property (Johnson 1999) and long setting time (Torabinejad et al. 1995, Dammaschke et al. 2005)[13]. Biodentine is one among these materials and is claimed to be used as a dentin restorative material in addition to its endodontic indications[14]. Appreciable properties of Biodentine includes, 1.superior physical properties 2. better handling properties 3. short setting time and 4. ability to stimulate tissue regeneration and exhibit good pulpal response[15].

The success of endodontic materials depends mainly on their ability to prevent leakage[16]. There are potentially two avenues by which leakage can occur at the apex of a root sealed with a retrograde filling. The first is by apical microleakage, that is leakage along the interface between the filling material and the canal wall. The second way is by the flow of fluids and substances along open tubules at the resected root end via permeable apical dentin. The sum of the leakage along these two pathways may be termed “apical leakage”[17]. Importance of apical seal in periradicular surgery defines the development of root-end filling materials[18.]

The importance of marginal adaptation is that it may have an indirect correlation with the sealing ability of retro-filling materials[19]. Among the various properties mentioned for retrograde filling material, marginal adaptation is very important for the success of an endodontic surgery (Stabholz et al., 1985; Peters and Peters, 2002)[20]. It is assumed that better the marginal adaptation of root end restorative materials, fewer irritants would pass through the interface between the filling materials and the root canal wall which is necessary for long term success[21].

An interface is a surface forming a common boundary for two different phases. In our study, interface indicates the surface between obturating material with root canal sealer and dentin, between root end filling material and dentin and between obturating material with root canal sealer and root end filling material. The interface plays a vital role in assessing the adaptability of root end filling material with the root dentin or the root end filling material with the root canal sealer. Interface evaluation between root end filling, root dentin and root canal sealer will give a better insight into marginal adaptation of root canal sealer and root end filling material with the dentin. Treatment outcome is affected by crack propagation and space in the interface between the material and the dentin walls[18].

Root canal sealer interface with dentin and root end filling material needs to be critically evaluated in the management of resected root end. The physical properties necessary for complete sealing of canal space include adaptation and adhesion of the filling material to the root canal wall, as gutta-percha does not directly bond to the dentin surface[22]. The main purpose of the root canal sealer is to fill the interface between the core material and the dentin wall, the voids inside the core material and the accessory canals, to serve as a lubricant and to obtain a hermetic apical seal[23]. Various root canal sealers are recommended, of these, AH Plus and Apexit Plus are widely used resin based and calcium hydroxide based root canal sealers respectively.

AH Plus is an epoxy resin based root canal sealer which can be used with gutta-percha to obtain a three dimensional restoration. AH Plus exhibits very low shrinkage during setting and has shown long term stability when compared to conventional sealers[24]. Apexit Plus is a calcium hydroxide based root canal sealer which is known for its property of stimulation of periapical tissues in order to maintain health or promote healing and secondly for its antimicrobial properties[25].

The degree of adaptation and quality of apical seal accomplished by root-end filling materials have been evaluated using dyes, radioisotopes, bacteria, scanning electron microscopy, electrochemical means and fluid filtration techniques[26]. Scanning electron microscope (SEM) is a powerful magnification tool that utilizes focused beam of electrons to generate an image. A highly detailed observation of the evaluated area is one of the main advantages of SEM[27].

So, the aim of this study was to evaluate the presence of gaps at three different interfaces between root canal sealer and root end filling material (sealer-dentin interface, root end filling material-dentin interface and sealer-root end filling material interface) using scanning electron microscope (SEM).

AIM:

The aim of this in vitro study was to evaluate the

i. Interface between Apexit Plus - dentin and AH Plus - dentin.
ii. Interface between MTA - dentin and Biodentine - dentin.
iii. Interface between Apexit Plus with MTA and Biodentine respectively, AH Plus with MTA and Biodentine respectively.

OBJECTIVE:

The objective of this study was to evaluate three different interfaces between root canal sealer and root end filling material (sealer-dentin interface, root end filling material-dentin interface, sealer-root end filling material interface) using Scanning Electron Microscope (SEM).

Min-Kai Wu et al[28] conducted an in vitro study in **1998** to measure the leakage of few root end filling materials in a longitudinal manner during a 1-year period using a fluid transport model. The roots of freshly extracted bovine central incisors were cut into root sections (3mm high). The root canals were machined to 2.6 mm in diameter. The central lumen of a hundred experimental root sections was obturated with zinc-free amalgam, Fuji II glass ionomer, Hi Dense glass ionomer, mineral trioxide aggregate (MTA) or Super-EBA and 20 root sections for each material. At 24 h, Fuji II leaked less than all of the other materials whereas MTA leaked more than amalgam, Hi Dense, or Super-EBA with no significant difference in leakage between them. At the 3 or 6months interval, amalgam leaked more than the other materials. Both Fuji II and MTA leaked less than Hi Dense and Super-EBA, whereas Hi Dense leaked significantly less than Super-EBA at the 6 month interval but this difference was less significant at the 3 month interval. At the 12-month interval, MTA, Fuji II, and Hi Dense leaked less than Super-EBA and amalgam. In summary, at 3-, 6-, and 12- month time intervals, both glass ionomer cements (Fuji II and Hi Dense) and MTA showed less leakage than the conventional amalgam and super-EBA, of which amalgam leaked more. Hence they concluded that the seal produced by MTA, was greatly improved during the first 3 months. Such improved seal was maintained until the end of the experiment. In presence of moisture, further hydration of MTA powder may result in an increase in compressive strength and a reduction in leakage.

E.Gondim, Jr et al[26] conducted an in vitro study in **2003** to compare the surface topography of root end after ultrasonic preparation, and again after root end fillings subjected to three different finishing techniques. The results revealed that eighty-nine percent of resected root ends presented marginal gaps around the root end fillings. The Super-EBA-filled roots finished with a Zekrya bur, displayed a significantly better dentine-root end filling interface adaptation when compared to the ball burnisher Super-EBA root end fillings. Super-EBA and IRM root end fillings finished with a ball burnisher or a Zekrya bur, showed significantly greater gap areas than roots filled with MTA. The root end filled with Super-EBA and finished with a ball burnisher displayed poorer marginal adaptation and presented the greatest average gap areas. They concluded that the marginal adaptation of MTA was good with or without finishing. Using a finishing bur over condensed and set IRM and super EBA provided better marginal adaptation.

C. Mangin et al[29] conducted an in vitro study in **2003** to determine whether hydroxyapatite cement (HAC) has a sealing ability that is comparable to commonly used, well studied retrofill materials, MTA and super EBA. Thirty of the prepared root sections were divided into 3 groups of 10 teeth each and each group was filled with one of the retrofill materials: MTA, HAC, or Super EBA. The results revealed that all the test materials leaked significantly compared with the negative controls and there was no significant differences found between the leakage rated of the three materials tested. They concluded that there is no significant differences in sealing ability between MTA, hydroxyapatite cement (HAC) or Super EBA.

Cristina Braga Xavier et al[30] conducted an in vitro study in **2005** to evaluate the root end sealing capacity and the marginal adaptation of MTA-Angelus, super EBA and Vitremer as root end filling materials as well as determine the existence of a correlation between apical microleakage and marginal adaptation in test materials. The results revealed that Vitremer presented higher microleakage than other groups, whereas MTA group leaked significantly less than Vitremer. SEM examination showed variable gaps between materials and dentin walls. MTA presented the smallest gaps and there were no statistical difference between super EBA and Vitremer. They concluded that the lack of gaps with root end filling materials/tooth interface did not hinder the dye penetration.

Eudes Gondim et al[21] conducted an in vitro study in **2005** to make a quantitative assessment of the sealing ability of super-EBA, IRM, and ProRoot MTA root end fillings subjected to 3 different finishing techniques. The results revealed that MTA displayed a significantly lower mean dye microleakage than EBA and IRM retrofillings. Root ends finished with a ball burnisher, although not differing significantly from those finished with Zekrya or 30-fluted carbide burs, displayed the greatest mean dye leakage. They concluded that the favorable results obtained with MTA in leakage studies may be related to its good marginal adaptation.

Necdet Adanir et al[31] conducted an in vitro study in **2006** to compare the sealing properties of different resin-based root canal sealers. Eighty extracted mandibular first premolar were selected and the canals were instrumented using profile 0.06 taper series 29 rotary instruments. The microleakage was tested using a fluid filtration device. Statistical analysis showed no significant differences among resin based sealers in terms of apical leakage (Group I-AH26, Group II-Endo REZ, and Group III-Diaket). However, zinc oxide– eugenol-based U/P Root Canal Sealer (Group IV) showed significantly more leakage than other groups. Hence they concluded none of the sealers used was completely able to prevent fluid leakage.

Zahed Mohammadi et al[32] conducted an in vitro study in **2006** to evaluate the sealing ability of Gray-MTA, White MTA and Resilon as root filling materials. The results showed no significant difference in leakage between GMTA and WMTA or between GMTA and Resilon. They concluded that the coronal seal produced by MTA preparations was similar to that produced by Resilon.

Maryam Bidar et al[33] conducted an in vitro study in **2007** to compare the marginal adaptation of white and grey MTA and Portland cement, using scanning electron microscopy (SEM). The results revealed no gaps between retrograde material and dentinal wall in 12% of the cases filled with white MTA. Sixty eight of the seventy five cases demonstrated some degree of gaps between filling material and canal wall. They concluded that both Grey and White MTA are suitable as root end materials. Given the low cost and similar sealing ability of the cements, it is reasonable to consider Portland cement as a possible substitute for MTA as a root end filling material.

Mohammad Ali Saghiri et al[16] conducted an in vitro study in **2008** to compare microleakage of MTA as a root end filling material in a solution at different pH values by using bovine serum albumin. The results revealed that the time needed for leakage to occur was significantly longer in samples stored in higher pH values. More porosity was observed on the surface of MTA exposed to lower pH values in the experimental groups. They concluded that, it might be advisable to use MTA with calcium phosphate cement(CPC) in situations in which MTA comes into direct contact with the lesions, not only as a matrix to control the placement of restorative materials but also as a material that is more resistant to an acidic environment.

Aline Tempel Costa et al[34] conducted an in vitro study in **2009** to evaluate the marginal adaptation of five root end filling materials; silver amalgam without zinc, white MTA-Angelus, white Portland cement (PC), Vitremer, and GC Fuji Ortho LC using Scanning Electron Microscope(SEM). The results showed a positive and significant correlation between marginal adaptation values of teeth and their replicas. They concluded that marginal adaptation and varying degrees of gap formation on the interface between dentin and root end filling material was found in all teeth.

Chun Cheng Chen et al[35] conducted an in vitro study in **2009** to examine the physicochemical properties of Al-free calcium silicate cements (CSCs). To investigate the phase composition, the specimens were ground to fine powders and then characterized with an x-ray diffractometer. Fourier transform infrared spectroscopy (FTIR) was used to analyze the powders. Scanning electron microscopy (SEM) was used to characterize the microstructure of the various specimens. The setting times for cements mixed with water ranged from 12–42 minutes and were lower for cements with higher starting calcium oxide content. Hence they concluded that the Al-free hydraulic calcium silicate cements exhibited shortened setting times and might prove the most useful for endodontic treatment requiring a setting time of a few minutes, such as root end filling/ sealing and pulp capping/cavity lining. But, further tests are necessary to confirm this statement.

U. Salz el al[23] conducted an in vitro study in **2009** to evaluate bacterial leakage of two root canal sealers, namely AH plus and Apexit plus by assessing the penetration of S.Mutans through coronally unsealed root canals. The results showed that AH plus had a slightly lower solubility (0.3% solubility) than Apexit Plus (0.5% solubility) and the film thickness of AH Plus was higher (28 µm) than Apexit Plus (11 µm). They concluded that better sealing ability of Apexit Plus compared with AH Plus may be explained by the physico-chemical properties and not by a potential antimicrobial effect of the material.

Leticia Kirst Post et al[36] conducted an in vitro study in **2010** to evaluate the effect of different apicoectomy instruments used in root end preparation, and dental materials used in retrofilling on apical sealing. The materials used for retrofilling were zinc-free silver amalgam or gray MTA. Based on dye penetration results, root end cavities were not completely sealed in any of the groups. The type of apicoectomy and instrument used in root end preparation were not significant factors. Teeth filled with gray MTA showed lower leakage values, independently of the combination of other factors. Hence, they concluded that the angle of apicoectomy and the type of root end preparation did not affect the degree of dye microleakage. The dental material used in retrofilling was the only factor significantly affecting microleakage results favoring the use of MTA.

Fernando Accorsi Orosco et al[37] conducted an in vitro study in **2010** to evaluate the sealing ability by dye leakage and the marginal adaptation by Scanning Electron Microscope (SEM), of apical plugs fabricated with gray MTA Angelus, CPM (brand name of a material similar to MTA) and a epoxy resin sealer containing calcium hydroxide (MBPC), as well as to verify the existence of a correlation between apical leakage and marginal adaptation in the tested materials. Results revealed significantly less apical leakage with MBPC than the other materials. SEM examination of the specimens showed multiple gaps between apical plugs and dentin walls. CPM presented the smallest gaps in extension, though without statistically significant from the other materials. Hence, they concluded that when used as apical plugs, the tested root end filling materials had similar marginal adaptation to the dentin walls, but MBPC had the best sealing ability, as demonstrated by the least apical leakage from all tested materials.

Thomas Von Arx et al[38] conducted a clinical study in **2010** to report the healing outcomes of 2 different methods of root end preparation and filling in apical surgery, MTA and an adhesive resin composite (Retroplast) patients undergoing apical surgery from May 2001-August 2007 were consecutively enrolled and a consent form from each patient were obtained. In healed cases, the radiograph demonstrated complete healing of the former radiolucency or incomplete healing, and no clinical signs or symptoms were present. In unhealed or non-healed cases, radiographic healing was assessed as uncertain or unsatisfactory, or clinical signs or symptoms were present, irrespective of the radiographic healing. The results revealed 85.5% overall rate of healed cases. MTA-treated teeth demonstrated a significantly higher rate of healed cases (91.3%) compared with Retroplast-treated teeth (79.5%). Within the MTA group, 89.5%–100% of cases were classified as healed, whereas it was 66.7%–100% in case of Retroplast group. They concluded that MTA can be recommended for root end filling in apical surgery, irrespective of the type of treated tooth. Whereas Retroplast should be used with caution for root end sealing in apical surgery of mandibular premolars and molars.

M. G. Gandolfi et al[39] conducted an in vitro study in **2010** aimed at gaining insight into the bioactivity of ProRoot MTA as a function of short soaking (up to 7 days) in phosphate solution standard disk samples of ProRoot MTA was prepared and immediately placed in DPBS at 37°C (Dulbecco's Phosphate Buffered Saline). After 7 days in DPBS, apatite deposits formed an evenly distributed layer on the entire surface. Punctual microanalyses and the elemental analysis on spherulites showed calcium (Ca) and phosphorus (P). FTIR analyses showed the formation of ettringite, portlandite, calcium silicate hydrate (CSH) phase and an apatite deposit after 5 hours immersion in DPBS. Hence, they concluded that the cement (ProRoot MTA) proved to be a sort of remineralizing agent and reservoir able to promote apatite deposition. It can also contribute to maintain a stable seal when placed in root end cavities and promote osteoblast growth.

M.H.Nekoofar et al[40] conducted an in vitro study in **2010** to investigate the effects of fresh human blood contamination on compressive strength and surface microstructure of grey ProRoot MTA and tooth colored ProRoot MTA. The surface microstructure of one extra specimen in each group was examined using scanning electron microscopy (SEM). In experimental groups in which MTA was mixed with water and exposed to blood, there was a significant difference in compressive strength between tooth-coloured MTA and grey MTA. They concluded that when further blood becomes incorporated in to MTA, the more the compressive strength of the material reduced. At the microstructure level, blood contamination of MTA resulted in a lack of acicular crystals resulting in reduction of compressive strength. Therefore, in clinical situations, in which blood becomes mixed with MTA, its physical properties are likely to be compromised.

PG Punitha et al[41] conducted an in vitro study in **2011** to evaluate and compare the adaptation of resin based sealers Epiphany, AH plus and AH26 to the root canal dentin using Scanning Electron Microscope (SEM). Statistical analysis showed that there are significant differences between the groups with respect to the mean gap and between the samples but interaction between the groups and the samples was not significant. They concluded that adaptation of epiphany sealer to dentin was best followed by AH plus and least adaptation was seen in AH 26, revealed by scanning electron microscope (SEM).

Silvio Taschieri et al[42] conducted study in **2011** to analyze the quality of root end filling in cases of periapical lesions persisting after endodontic surgery. Ten patients requiring extraction, who had been previously subjected to endodontic surgery, performed with a modern technique and using zinc oxide EBA reinforced cement as the root end filling material. Soon after the extraction procedure, an impression of the resected root surface was obtained with polyvinylsiloxane material and subjected to SEM analysis. The results showed gaps between the material and canal walls in all the root end fillings. They concluded that this defective apical seal would favour continuous leakage of surviving bacteria and their by-products from the infected root canal to periapical tissues leading to persistence of inflammation.

David c. Bird et al[43] conducted an in vitro study in **2012** to compare the ability of Capasio and MTA to penetrate human dentinal tubules when used as a root end filling material and to examine the interaction of capasio and MTA with synthetic tissue fluid (STF) and endodontically prepared root canal walls in extracted human teeth. The results showed Penetration of Capasio into dentinal tubules in 17 out of 35 samples, whereas there is no penetration of MTA into dentinal tubules was observed at any level. Both Capasio and MTA formed apatite crystals in the supernatant, on their exposed surfaces, and in the interfacial layers that were similar in structure and elemental composition when evaluated by using SEM. They concluded that, when used as a root end filling material, Capasio is more likely to penetrate dentinal tubules and equally likely to promote apatite deposition when compared with MTA.

Joao Eduardo Gomes-Filho et al[44] conducted an in vitro study in **2012** to evaluate the apical sealing ability of Fillapex, Endo-CPM sealer and Sealapex. Statistical analysis showed that Endo-CPM Sealer allowed more leakage than the other materials. Fillapex was similar to Sealapex and both materials showed significantly less leakage when compared to Endo-CPM-Sealer. They concluded that Sealapex and Fillapex were able to significantly present apical leakage when compared to Endo-CPM sealer, which showed highest levels of leakage.

Sharad R.Kokate et al[45] conducted an in vitro study in **2012** to compare the microleakage of three different root end filling materials Glass Ionomer Cement (GIC), Mineral Trioxide Aggregate (MTA), & Biodentine using dye penetration method under a stereomicroscope. Based on the results, the microleakage was found to be significantly less in Biodentine. Hence they concluded that GIC, MTA and Biodentine exhibited microleakage with Biodentine showing the least microleakage of all.

Grech et al[46] assessed in **2013** the composition of materials and products of a prototype cement of tricalcium silicate and radiopacifier and two commercially available tricalcium silicate cements, one of which was Biodentine. They concluded that Biodentine resulted in the formation of calcium silicate hydrate and calcium and hydroxide which leached in solution. The materials, when hydrated, consisted of a cementitious phase, rich in calcium, silicone, and a radiopacifying material. Biodentine was further described as having calcium carbonate in powder and the carbonate phase of the material was verified by X-ray energy dispersive analysis (XRD) and Fourier transform infrared spectroscopy (FTIR) analysis.

Hui-min Zhou et al[47] conducted an in vitro study in **2013** to evaluate the pH change, viscosity and other physical properties of 2 novel root canal sealers (MTA Fillapex and Endosequence BC) in comparison with 2 epoxy resin-based sealers (AHPlus and ThermaSeal), a silicone-based sealer (Gutta- Flow), and a zinc oxide-eugenol–based sealer (Pulp Canal Sealer). The results revealed that the MTA Fillapex sealer exhibited a higher flow than the Endosequence BC sealer. The MTA Fillapex and Endosequence BC sealers showed the highest film thickness among the tested samples. The MTA Fillapex and Endosequence BC sealers presented an alkaline pH at all times. The pH of fresh samples of the AH Plus and ThermaSeal sealers was alkaline at first but decreased significantly after 24 hours. The viscosity of the tested sealers increased with the decreased injection rates. They concluded that the tested endodontic sealers are pseudoplastic, so that their viscosity. The new endodontic sealers, MTA Fillapex and Endosequence BC, each possessed comparable flow and dimensional stability but higher film thickness and solubility than AH Plus, ThermaSeal, PCS, and GuttaFlow.

Seda Aydemir et al[48] conducted an in vitro study in **2013** to evaluate and compare root end surfaces for the presence of cracks after root end cavity preparation using zirconium nitride coated Ultrasonic (US) retrotips and Er: YAG laser. No statistically significant difference was detected between the US and laser groups for complete, incomplete, intradentinal and total number of cracks. Hence statistical analysis revealed no significant effect of retropreparation technique in the development of apical cracks.

Vivek Aggarwal et al[49] conducted an in vitro study in **2013** to comparatively evaluate the push-out bond strength of ProRoot MTA, Biodentine, and MTA plus in repairing furcal perforations utilizing an internal matrix, when subjected to blood contamination.

The results revealed significant increase in the push-out bond strength of samples with increase of setting time (from 24 h to 7 days). Blood contamination affected the MTA samples with a setting time of 7 days, but had no significant effect on 24-h samples. In the MTA Plus group, blood contamination significantly decreased the strength both in 24-h and 7-days samples, whereas it had no effect on the perforations repaired with Biodentine. In 24-h samples, MTA had significantly less push-out bond strength than Biodentine and MTA Plus whereas in 7-days group, MTA Plus had significantly less push-out strength than the other repair materials. They concluded that all samples, irrespective of setting time or contamination status, had shown a dislodgement resistance more than the average force recorded during amalgam condensation.

Han and Okiji[50] conducted an in vitro study in **2013** to compare the ability of white root MTA, Endosequence BC sealer and Biodentine to produce apatites and cause calcium and silicate incorporation in adjacent human root canal dentin after immersion in phosphate buffered saline (PBS). Root sections of human single rooted teeth were filled with one of the materials and immersed in PBS for 1, 7, 30 or 90 days. The results revealed that all materials produced surface precipitates of acicular or lath-like morphology with Ca/P ratio of 1.6 : 2.0. Within dentinal tubules, the three materials formed tag-like structures that were frequently composed of Ca- and P- rich and Si-poor materials, suggesting intratubular precipitation. Ca- and Si-incorporation depths were in the order of Biodentine followed by WMTA followed by BC sealer, with a significant difference between BC sealer and the others at several time points. Hence, they concluded that when compared with Biodentine and WMTA, BC sealer showed less Ca ion release and did not show Ca and Si incorporation as deeply in human root canal dentin when immersed in PBS for up to 90 days.

Ayush Razdan Singh et al[51] conducted an in vitro study in **2013** to evaluate the microleakage of various root end filling materials and to find the sealing ability of these materials. They concluded that the mean microleakage of group B (Super EBA) was minimum when compared to other group with Bone Cement, Glass Ionomer and MTA.

Helder Fernandes Oliveirae et al[18] conducted an in vitro study in **2013** to evaluate the marginal adaptation of root end filling materials using Scanning Electron Microscope (SEM). The root end cavities were retrofilled with the respective root end materials. Based on SEM images, the results revealed the specimens with ProRoot MTA had no significant difference in marginal adaptation compared to those with IRM, amalgam, Super-EBA and Epiphany/Resilon groups. Hence they concluded that all the above tested materials showed similar marginal adaption when used as root end filling.

Jale Tanalp et al[52] conducted an invitro study in **2013** to comparatively evaluate the radiopacity values of 3 root end filling materials, Biodentine, MM-MTA and MTA Angelus. The results revealed higher radiopacities with all materials tested compared to the control (dentin) in their respective groups. Hence they concluded that, though all materials had higher radiopacities compared to dentin, the relatively lower radiopacity of Biodentine compared to other materials can be improved to achieve more reliable results in root end filling procedures.

Pedro Felício Estrada Bernabé et al[53] conducted an in vitro study in **2013** to evaluate the sealing ability of MTA root end-filling material placed using three techniques: 1. hand condensation with indirect sonic activation; 2. hand condensation with indirect ultrasonic activation; and 3. hand condensation without indirect activation. The results revealed that the indirect sonic activation of MTA resulted in a better sealing ability with lowest infiltration values when compared with hand condensation alone or ultrasonic activation. They concluded that sonic vibration could be considered as an efficient aid to improve the sealing ability of MTA when used as root end filling material.

R.A.Rosa et al[27] conducted an in vitro study in **2013** to evaluate, by scanning electron microscopy, the presence of gaps at the interface between filling material and three root end filling materials. The results revealed highest gap areas recorded for Super EBA and the lowest for MTA, with statistically significant differences. They concluded that super EBA, MTA, and Portland cement presented greater adaptation to the root canal filling despite the statistical differences observed between super EBA and MTA.

Y-Z. Chen et al[54] conducted an in vitro study in **2013** to develop a novel root end filling material (NRFM) based on hydroxyapatite titracalcium phosphate, and polyacrylic acid, to determine its chemical composition and to compare its physical properties and cytotoxicity with those of Glass Ionomer Cement (GIC) and Portland cement (GPC). Based on results, GIC had significantly greater compressive strength than both NRFM and GPC at various time intervals. NRFM storage solution showed an initial weak acidity and then gradually increased to a weak alkalinity, with pH values ranging from 6.14 to 8.28; the GIC was acidic and GPC was alkaline. The washout resistance of NRFM was superior to those of GIC and GPC. They concluded that the novel root filling material (NRFM) possessed suitable physical properties in terms of setting time, washout resistance and pH, compared with GIC and GPC.

Andiara De Rossi et al[55] conducted an clinical study in **2014** to evaluate pulpal and periapical response of dogs teeth after pulpotomy and pulp capping with Biodentine and MTA by radiographic, histopathologic, and histo-microbiological analysis. Histopathologic and histomicrobiological analyses revealed mineralized tissue bridge formation, pulpal vitality, odontoblast layer integrity, preserved periodontal ligament, and absence of bone or root resorption and microorganisms in both groups. Although the bridges formed at the amputation site had similar morphology, they were significantly thicker in the Biodentine group. They concluded that Biodentine presented tissue compatibility and allowed for mineralized tissue bridge formation after pulpotomy in dog's teeth in all specimens with similar morphology and integrity to those formed with use of MTA.

Graziela Garrido Mori et al[56] conducted a animal study in **2014** to evaluate the biocompatibility of Biodentine in the subcutaneous tissue of rats. The study was conducted on 15 male rats. According to the results, the analysis of the histologic control sections confirmed the biocompatibility of the tubes with the connective tissue. In all the study periods, the inflammatory process was not significant or mild in the connective tissue in contact with the empty tube and the tube containing zinc oxide eugenol. Microscopic analysis of the histologic sections confirmed the irritability of zinc oxide eugenol to the connective tissue showing moderate to severe inflammatory process. The connective tissue was moderately inflamed at 7 days when in contact with Biodentine, whereas at 14 and 30 days, the inflammatory process was mild or not significant. They concluded that the Biodentine showed an initial inflammatory response, but that response was quickly followed by biocompatiable acceptance of Biodentine by the contacted tissue after 2weeks.

Ravichandran P.V. et al[57] conducted an in vitro study in **2014** to evaluate the marginal adaptation of three root end filling materials: Glass ionomer cement, Mineral trioxide aggregate and Biodentine. Statistical analysis showed lowest mean gap area found in Biodentine followed by MTA and GIC. They concluded that this new root end filling material Biodentine showed better marginal adaptation than commonly used root end filling materials.

Seok-Woo Chang et al[58] conducted an in vitro study in **2014** to investigate the biocompatibility, inflammatory response, and potential for odontoblastic differentiation of Biodentine compared with Ortho-MTA, Angelus-MTA, and intermediate restorative material (IRM) in human dental pulp cells. The results showed similar levels of cell viability for Prodentine, OMTA and AMTA compared with the osteogenic supplement-treated groups at 3, 7 and 14 days. The formation of mineralized modules and alkaline phosphatase activity (ALP) were increased with AMTA and Prodentine, but decreased with IRM. The levels of intracellular reactive oxygen species (ROS) were similar in HDPCs exposed to Biodentine, OMTA, or AMTA. . Hence they concluded that, for the first time Prodentine exhibited similar biocompatibility, inflammatory response, and odontoblastic differentiation compared with OMTA and AMTA in HDPSC, which suggests that Biodentine can be used for pulp capping agent.

Zhirong Luo et al[59] conducted an in vitro study in **2014** to investigate the effect of Biodentine on the response of human dental pulp stem cells (hDPSCs) in terms of proliferation, migration, adhesion and the involvement of different chemokines and adhesion molecules in cultured hDPSCs. They concluded that Biodentine to be a bioactive and biocompatible material capable of enhancing hDPSCs proliferation, migration and adhesion abilities.

Naziya Butt et al.[60] conducted an invitro study in **2014** to compare at different times, the sealing ability, calculated as a fluid filtration rate of roots filled with two commercially available calcium silicate cements: Biodentine and white MTA(WMTA) and evaluation of their initial setting time, handling properties and compressive strength. Statistical analysis of the fluid filtration results revealed significant differences in microleakage amongst the two root end filling materials examined at 4 and 24 h storage periods and no difference at 1, 2, 4, 8, and 12 weeks. Compressive strength of Biodentine was significantly higher than MTA-Angelus. They concluded that the sealing quality of Biodentine and commercially available MTA cement (MTA‑Angelus) is comparable. The enhancement in handling properties of Biodentine may make it more convenient for use in various clinical applications.

Neha Sharma et al[61] conducted an in vitro study in **2014** to evaluate the marginal adaptation of PMMA Bone cement, MTA and Amalgam as root end filling materials. SEM examination of the interface showed that bone cement group had the least gap measurements, whereas maximum gaps were found in samples filled with amalgam. All the retrograde root fillings showed leakage with dye penetration test. Although none had a score of zero, the scores were less in MTA and Bone Cement than those for amalgam. They concluded that bone Cement and MTA exhibited a better adaptation to the dentinal walls than Amalgam.

Saravanapriyan Soundappan et al[19] conducted an in vitro study in **2014** to compare the marginal adaptation of Biodentine with MTA and Intermediate restorative material (IRM) using scanning electron microscope (SEM). The results revealed that the mean gap at the dentin- retrograde filling material interface was maximum for Biodentine followed by IRM and MTA. They concluded that the marginal adaptation of MTA and IRM were superior to Biodentine.

R Gnana Seelan et al[62] conducted an in vitro study in **2015** to compare five different root canal sealers against E.Faecalis in an infected root canal model by using real time PCR (RT-PCR). The results revealed maximum antibacterial activity was achieved with Tubli-Seal and least by RoekoSeal. They concluded that the maximum antimicrobial activity against E.Faecalis was achieved with AH-plus and Tubli-seal and the least with RockoSeal. RT-PCR can be used as a valuable and accurate tool for testing antimicrobial activity of root canal sealers.

Alicja Nowicka et al[63] conducted an clinical study in **2015** aiming at tomographic evaluations of reparative dentin bridges formed after direct pulp capping with Ca(OH)2, MTA, Biodentine, and Single Bond Universal in human teeth to verify the null hypothesis that there is no difference in the quantity and quality of reparative dentin formation between the evaluated materials used for direct pulp capping in human teeth. Ca(OH)2, MTA, and Biodentine actively initiated the formation of reparative dentin in each tooth, whereas single bond universal was less active and induced the formation of 2 small and 2 very small bridges. The reparative hard tissues in Ca(OH)2 and single bond universal groups had an uneven thickness and exhibited porosities and tunnel defects, whereas those in the MTA and Biodentine groups were thicker and more homogeneous with minimal tunnel defects. They concluded that the volume of formed reparative dentin bridges depend upon the material used for direct pulp capping. Biodentine and MTA induced the formation of bridges with a significantly higher average volume compared with single bond universal and CBCT imaging allowed for the identification of the location of dentin bridges.

Bhalla S Ajeet et al[64] conducted an in vitro study in **2015** to evaluate and compare the sealing ability of four different retrograde filling materials MTA, IRM, Silver Amalgam and Giomer with or without root end bevel using dye penetration technique under stereomicroscope: when the root ends were resected at two different angles (45 degrees and 0 degrees). 90 extracted human maxillary central incisor were selected. The results showed mean microleakage values that were higher with use of bevel as compared to those without the use of bevel. Intergroup comparison revealed that MTA without bevel had minimum and Giomer with bevel had maximum leakage. They concluded that use of MTA as root end filling material without bevelling the root end (0º bevel) may be considered as a procedure of choice for predictable clinical results.

Ian Chen *et al*[65] conducted an animal study in **2015** to compare healing after root end surgery by using Gray MTA and ERRM as root end filling material in an animal model where apical periodontitis was induced in 55 mandibular premolars of 4 healthy beagle dogs. After 6 weeks, microsurgical root end surgeries were performed. MTA and ERRM were used as root end filling materials. After 6 months, when healing of the periapical area were assessed using periapical radiographs, cone beam computed tomography and microcomputed tomography, it was found that ERRM was a biocompatible material similar to MTA with good sealing ability.

Khalid Al Fouzan et al[66] conducted an in vitro study in **2015** to compare the marginal adaptation of MTA to root dentin between orthograde and retrograde application techniques and also to assess the effect of 17% EDTA on the MTA root dentin adaptation using microcomputed tomography (micro-CT) analysis. . Fifty-two single-rooted human teeth were selected and divided into four equal groups according to proposed root-end filling procedures: 1. Retrograde MTA (RMTA), 2. Orthograde MTA (OMTA), 3. Etched RMTA (ERMTA), and 4. Etched OMTA (EOMTA). The results revealed no significant difference observed in the dentin-MTA adaptation for orthograde and retrograde application technique. Hence, they concluded that MTA adaptation to dentin tooth structure was not significantly different between an orthograde and retrograde approach. However, the use of EDTA significantly improved the MTA-dentin adaptation.

Salin Nanjappa A et al[67] conducted an in vitro study in **2015** to compare the sealing ability of MTA, Biodentine, Chitra-CPC when used as root end filling and to evaluate effect of ultrasonic retrotip and an Er:YAG laser on the integrity of three different root end filling materials. The amount of dye penetration was found to be lesser in root ends prepared using Er:YAG laser when compared with ultrasonics with statistically significant difference. They concluded that Biodentine is a better material to prevent apical microleakage in comparison to MTA and Chitra-CPC. Root end preparation with Er:YAG laser exhibited lesser amount of dye penetration when compared to ultrasonics, which may be due to absence of chipping during root end cavity preparation with Er:YAG Laser.

Swati A. Borkar[68] in **2015** reported for cases of traumatized, fully matured, maxillary permanent central incisors, which have been treated by Biodentine pulpotomy several days after traumatic pulp exposure. The results revealed absence of tenderness to percussion and the teeth were asymptomatic in which pulpotomy was carried out. Electric pulp testing revealed vital response in all the four teeth treated using Biodentine pulpotomy at the end of 18 months. Radiographic examination showed absence of periapical lesion or widening. Hence they concluded that the favorable results of the current cases suggesting the interval between trauma and treatment are not critical for pulp recovery provided that the pulp is vital, the superficially inflamed tissue is removed, and a proper aseptic procedure is performed using bio-compatible materials without additional pulp stress. Biodentine pulpotomy can be recommended as a treatment option for cases of vital pulp exposure in permanent incisors due to trauma.

Anurag Jain et al[69] conducted an in vitro study in **2016** to compare the sealing ability of four root end filling materials MTA, Portland cement, IRM, Resin modified glass ionomer cement (RMGIC) in teeth with root apices resected at 0º and 45º angle using dye penetration method under fluorescent microscope. The results revealed that the root apex sealing ability of MTA was superior to Portland cement, IRM and light cure GIC. IRM demonstrated the maximum apical leakage value among all the materials. Portland cement and Light cure GIC (LC-GIC) showed comparable sealing ability. Hence they concluded that microleakage was observed in all the subgroups. MTA recorded the least apical leakage value while IRM recorded the maximum apical leakage value among all the materials. There was no statistically significant difference among all the materials at 0° and 45° angle.

Dennis Tran et al[70]conducted an in vitro study in **2016** to compare the marginal adaptation of ProRoot MTA, Neo-MTA plus, and endosequence BC RRM-fast set putty after orthograde placement in roots with open apices. The results showed no significant differences between the 4 experimental groups in the quality of the marginal adaptation of the root filling material at the level of the anatomic apex. They concluded that all materials showed comparable marginal adaptation at the anatomic apex when used for orthograde obturation of open apices. Application of BC sealer before the delivery BC RRM-FS putty enhanced the quality of adaptation coronal to the apex.

S.no	Material used	Brand name/Manufacture details
1.	Human maxillary central incisors	n=80
2.	Endodontic access bur	Dentsply Maillefer, Ballaigues, Switzerland
3.	Gates glidden drills No. 1 to 3	Mani, Japan
4.	ISO size 10 to 40 K-Files	Mani, Japan
5.	17% EDTA	Prime Dental Products, India
6.	5.2% sodium hypochlorite	Asian Acrylates, India.
7.	Normal saline	Claris Otsuka Private Limited, India.
8.	Paper Points	Hygienic, Coltene, USA
9.	Lentulospiral	Dentsply Maillefer, Balllaigues, Switzerland
10.	AH Plus Resin Sealer	Dentsply, Newyork, USA
11.	Apexit Plus Sealer	Ivoclar Vivident AG, Schaan, Liechtenstein

12.	Guttapercha Points Size 15 to 40	Diadent International, Canada
13.	Diamond disc	Axis dental, Kavo Kerr, Germany
14.	Graduated periodontal probe	Hu-Friedy, USA
15.	MTA	Prevest Denpro, India
16.	Biodentine	Septodont, Saint Maur, France
17.	MTA carrier	Dentsply, Tulsa dental, USA
18.	Non standardized hand plugger	GDC dental, India

S.no	Equipments used	Brandname / Manufacturer details
1.	Ultrasonic scaler	Satelec, Acteon, France
2.	Ultrasonic tips	P14D, Satelec, Acteon, France
3.	Micromotor handpiece	NSK pana-max plus, Nakanishi International, Tokyo
4.	Radiovisiography (RVG)	Satelec, Acteon, France
5.	Scanning Electron Microscope(SEM)	Vega3, Tescan, USA

After approval of the Ethical committee of our college, eighty extracted human maxillary incisors with mature apices, free of caries and without root fracture or resorption were selected for this study (Fig 1.A). The selected teeth were cleaned with ultrasonic scaler to remove any soft tissue or calculus covering the roots and were stored in saline solution until the preparation. The crowns of the teeth were sectioned transversely to create a standardized tooth length of 16 mm from the root apex (Fig 1.B) with a diamond disc mounted to a slow speed straight handpiece under continuous water spray (Fig 1.C).

Root canal preparation:

After taking initial radiographs, the cervical third of each root canal was enlarged using Gates-Glidden drills, sizes 1 to 3 (Mani, Inc, Japan). The working length was established by introducing ISO size 10 K file (Mani, Inc, Japan) (Fig 2.A) into the root canal until it was visible at the apical foramen and then subtracting 1mm from this length. The canals were instrumented to the working length up to size 40 K file (Mani, Inc, Japan) using step back technique (Fig 2.B). The canals were irrigated with 5% sodium hypochlorite followed by saline rinse between every instrument change. The final irrigation was done using 17% EDTA (Prime Dental Products India) followed by saline and were then dried with paper points.

The teeth were randomly divided into two groups of forty samples each as,

Group I (n=40)

The root canals were coated with Apexit plus sealer (Ivoclar Vivadent) (Fig 2.E), with a lentulospiral and were obturated with 2% guttapercha points with size 40 as master cone

(Fig 2.C) followed by accessory cones using cold lateral condensation technique (Fig 2.D).

Group II (n=40)

The root canals were coated with AH plus sealer (Ivoclar Vivadent) (Fig 2.F), with a lentulospiral and were then obturated with 2% gutta-percha points with size 40 as master cone (Fig 2.C) followed by accessory cones using cold lateral condensation technique (Fig 2.D).

Root end preparation and retro filling:

Roots were resected perpendicular to the long axis of the root with a diamond disc at 3mm from the apex (Fig 3.A) using a straight handpiece with continuous water spray (Fig 3.B). Root end cavities were made in all teeth using an ultrasonic retro preparation diamond tip (P14D, Satelec, France) (Fig 3.C) attached to a ultrasonic scaler unit (Satelec, P5 Newtron, Acteon, France) (Fig 3.D) to a depth of 3mm (Fig 3.E).The depth of the cavity was checked using a graduated periodontal probe (Fig 3.F) to standardize the retro preparation (Fig 3.G & Fig 3.H).

Sample grouping:

The two groups, Group I and Group II were further subdivided into four subgroups of twenty samples each as, n=80

- Group I (Apexit Plus) (n=40)
  - Subgroup A (MTA) (n=20)
  - Subgroup B (Biodentine) (n=20)
- Group II (AH Plus) (n=40)
  - Subgroup C (MTA) (n=20)
  - Subgroup D (Biodentine) (n=20)

Both the root end materials, MTA (Fig 3.I) and Biodentine (Fig 3.J) were manipulated according to manufacturer's instructions. The mixed material was dispensed into the retro cavity using a root end filling material MTA carrier (Fig 3.K) and condensed into the cavity using a hand plugger (Fig 3.L, 3.M and 3.N) according to 4 subgroups divided (Fig 3.O, Fig 3.P, Fig 3.Q and Fig 3.R)

All the teeth were wrapped in a wet gauze and placed in an incubator at 37ºC for 24 hours for the root end filling material to set completely (Fig 3.S).

Scanning Electron Microscopy (SEM):

The roots were transversally sectioned with a diamond disc using a straight handpiece with water coolant to obtain blocks, 6 mm in length containing the apical third (Fig 4.A).

The blocks were again longitudinally sectioned (Fig 4.B) to expose the interface between the sealer and root end filling material (Fig 4.C).

The specimens were then placed on metal stubs and properly labeled (Fig 5.A). The specimens were gold sputtered (Fig 5.B), which is a process of coating the specimens with a thin layer of conducting material, typically a metal such as gold, for enhancement of electrical conductivity of the samples. They were then placed and viewed under SEM (Vega3 Tescan, USA), operating at 10KV under 600x magnification (Fig 5.C and Fig 5.D). All the three interfaces (Fig 5.E), root canal sealer – dentin interface, root end filling material – dentin interface and root canal sealer – root end filling material interface were viewed.

SEM Analysis and Statistics:

Initially, the electron micrographs were obtained with 100 x magnification to view all the three interfaces (root canal sealer-dentin interface, root end filling-dentin interface and GP with root canal sealer-root end filling material interface). Next, the presence of gaps at the interfaces was assessed by quantitative measures in micrometers (µm) using SEM with 600 x magnification. Two widest gap distance measurements (µm) were taken for each interface and the mean values and standard deviation were calculated.

Statistical analysis was performed by SPSS software package, Version 20.0 (Microsoft, IL, USA). Data were analyzed using Mann Whitney test for root canal sealer-dentin and root end filling material-dentin interfaces. Interface at root canal sealer and root end filling material was analyzed using Kruskal Wallis test followed by Mann Whitney test with $p<0.05$ level of significance.

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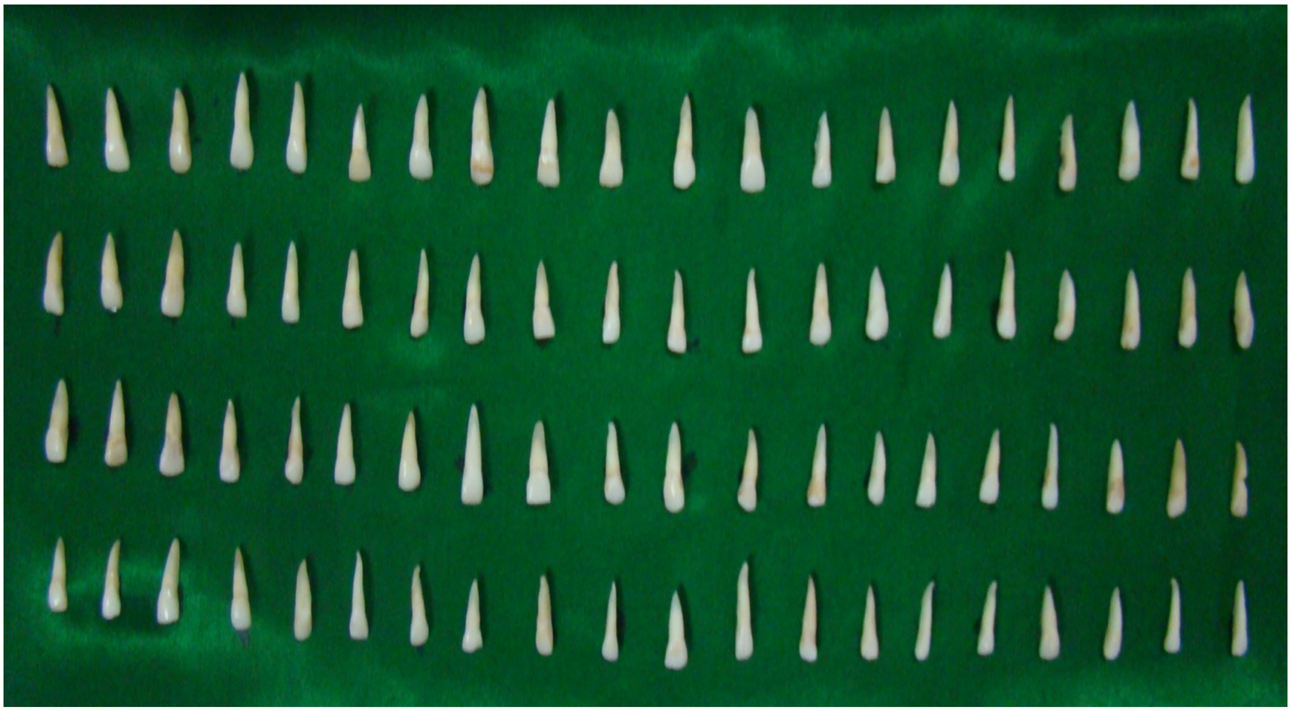

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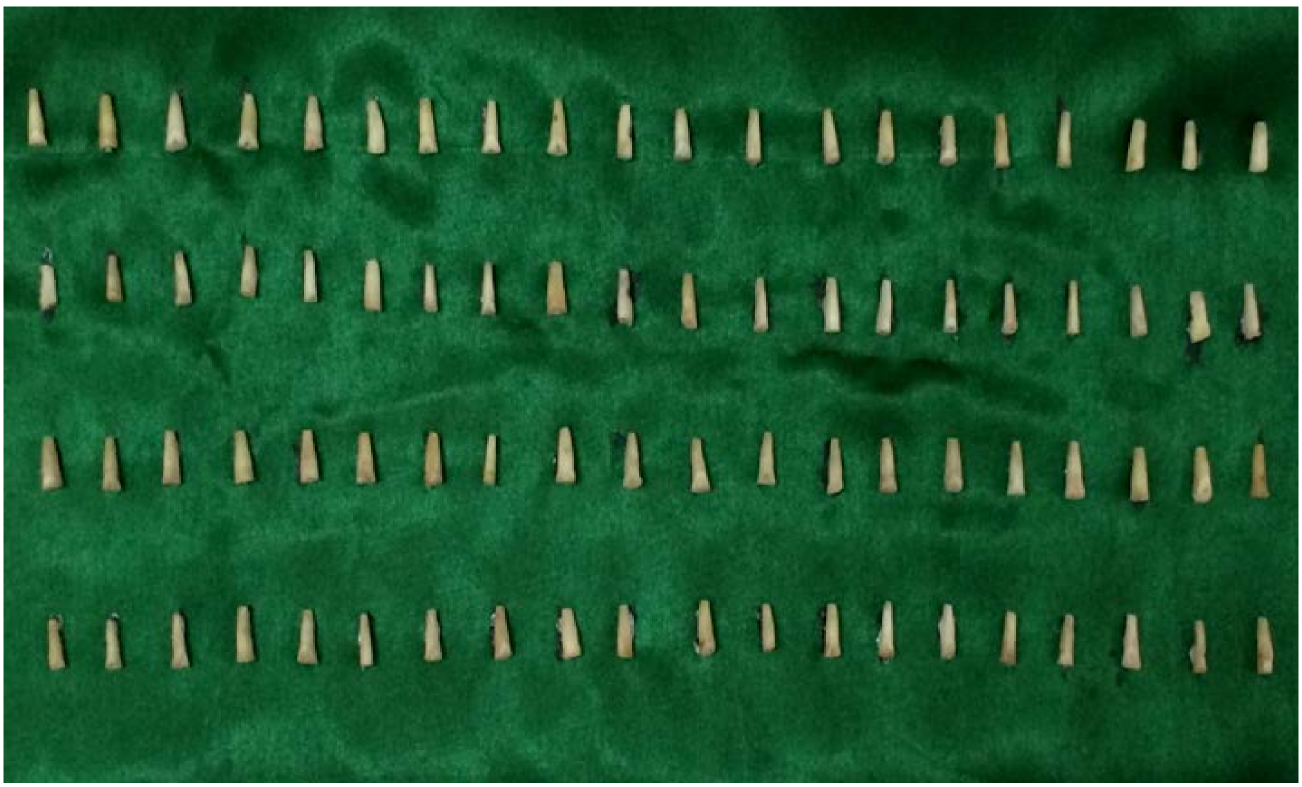

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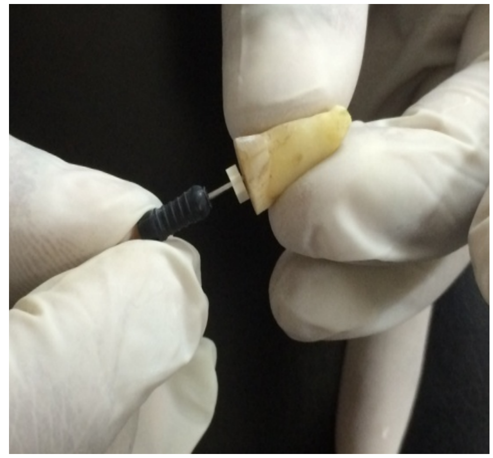

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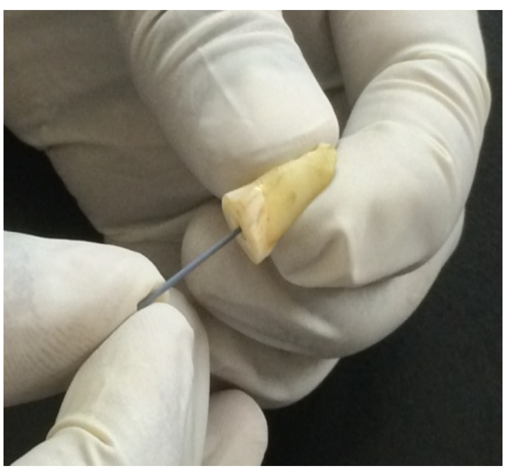

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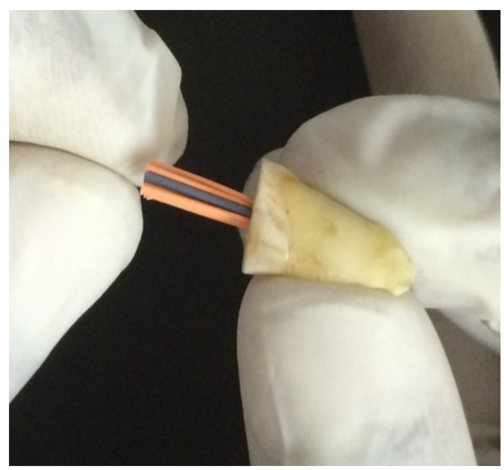

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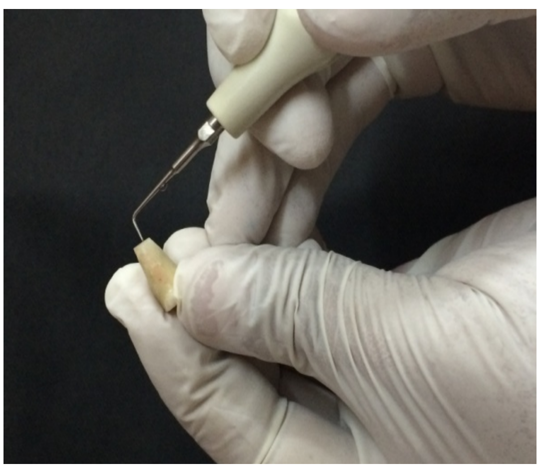

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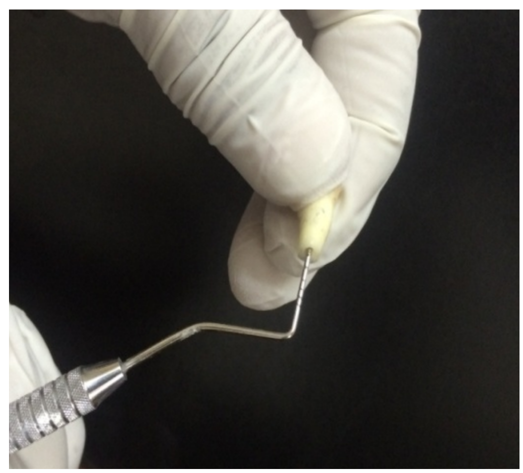

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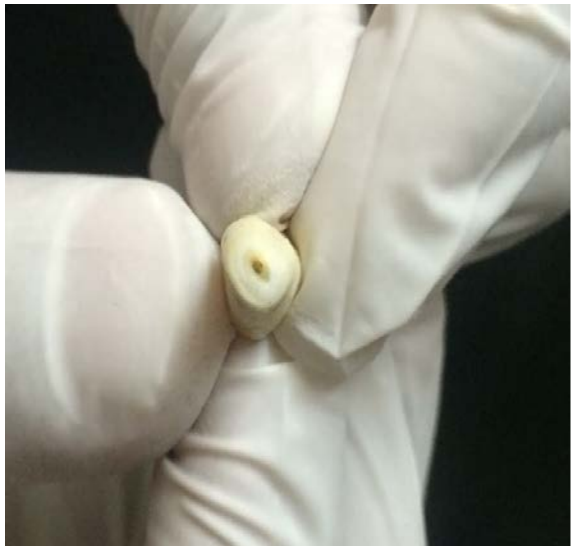

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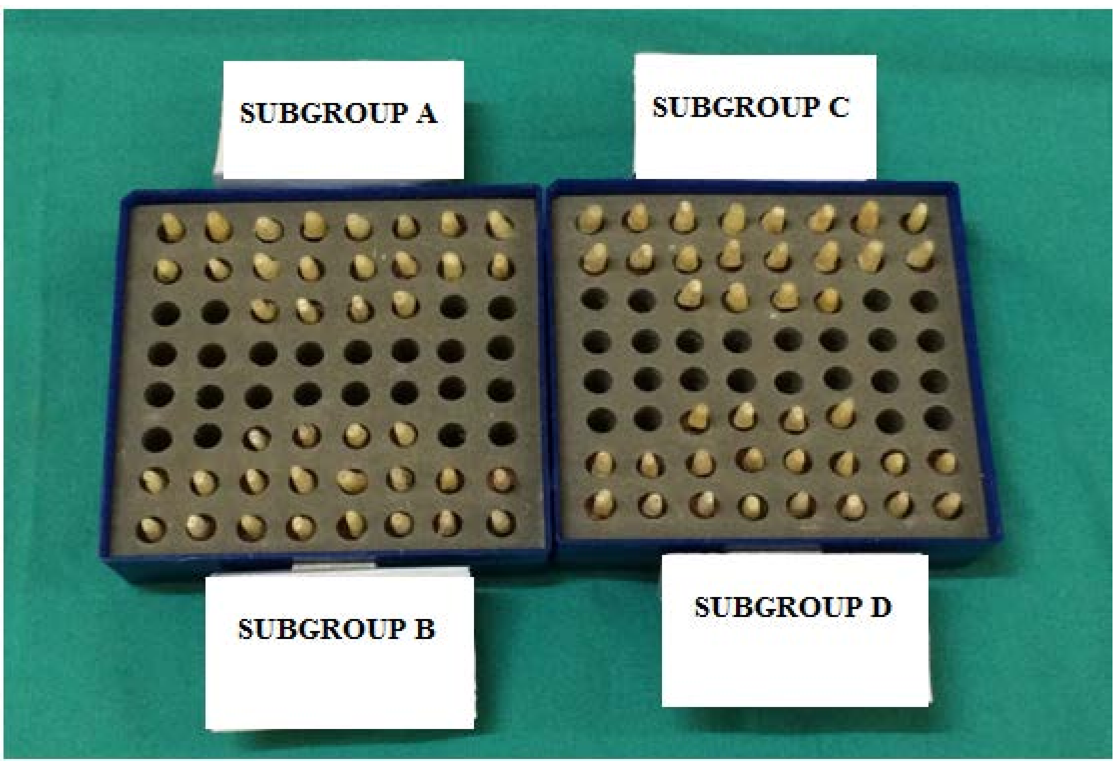

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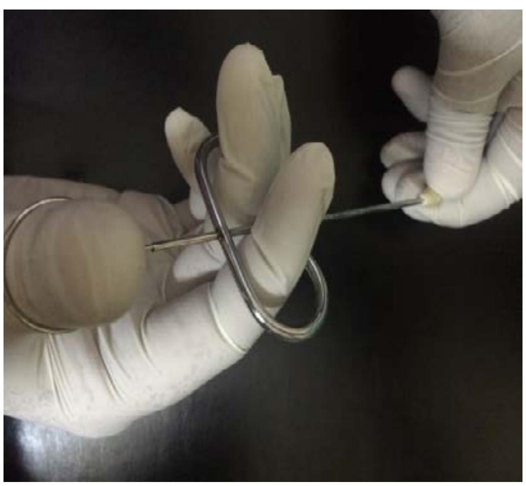

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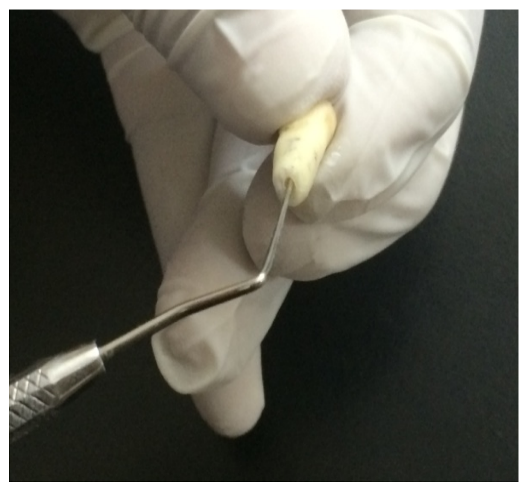

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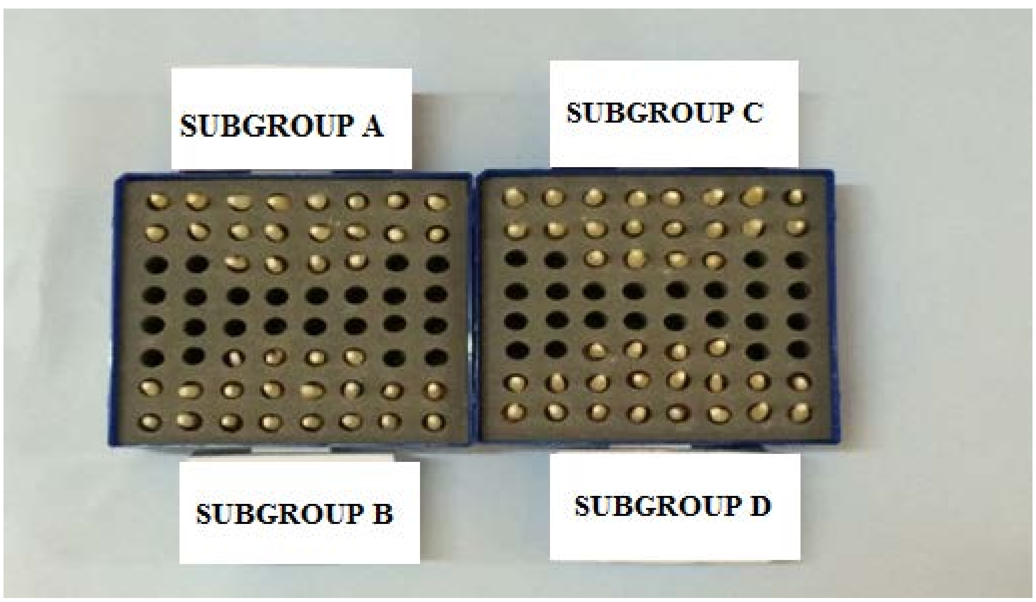

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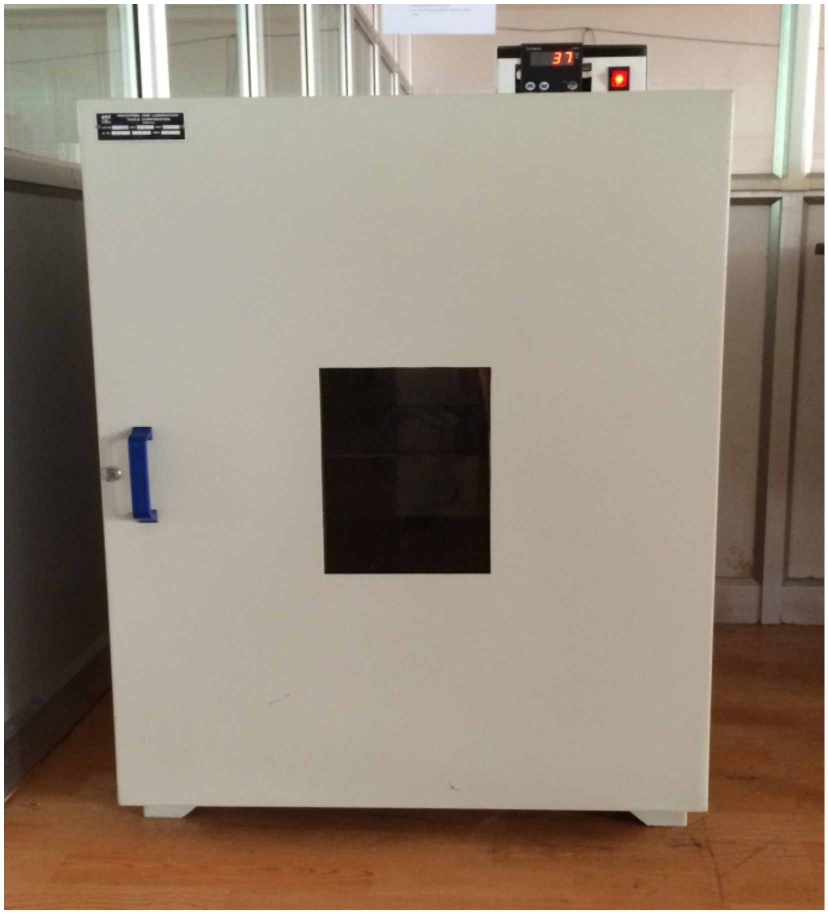

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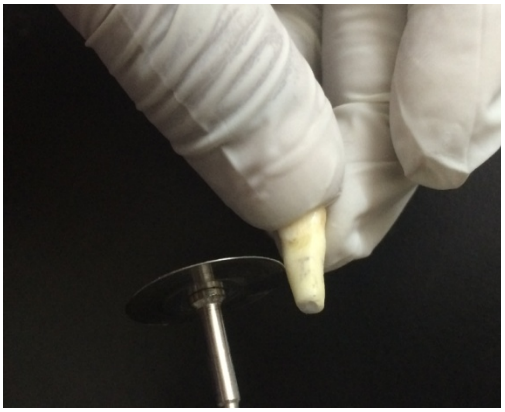

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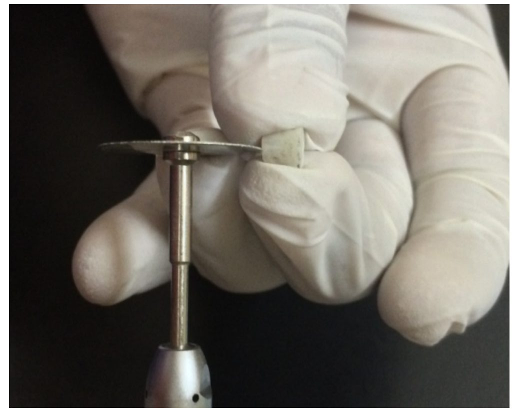

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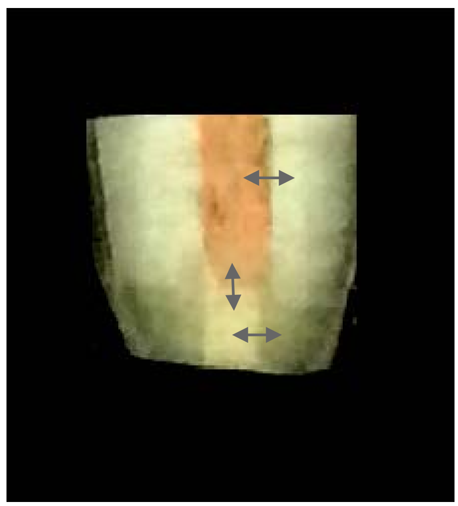

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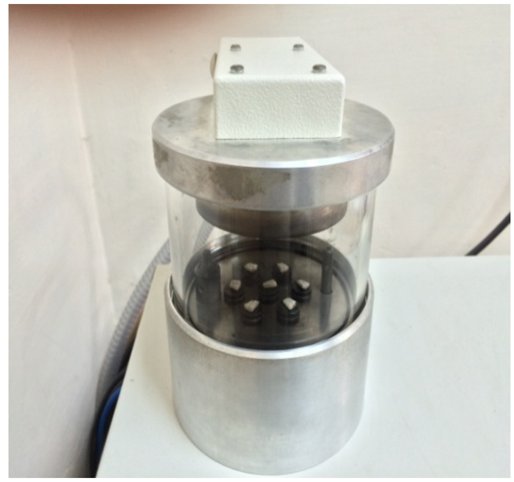

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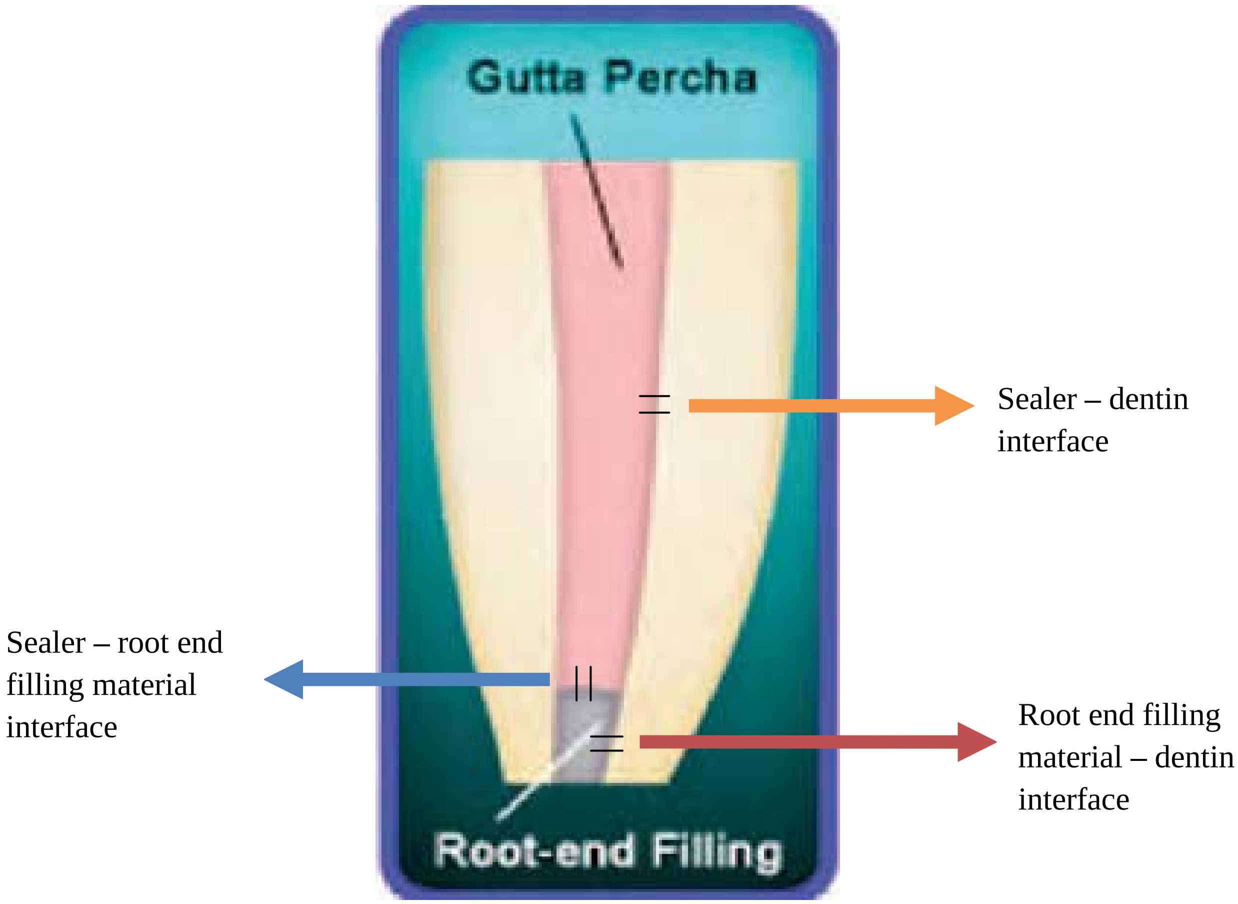

Fig 5.E: Diagrammatic representation of teeth sample showing three interfaces

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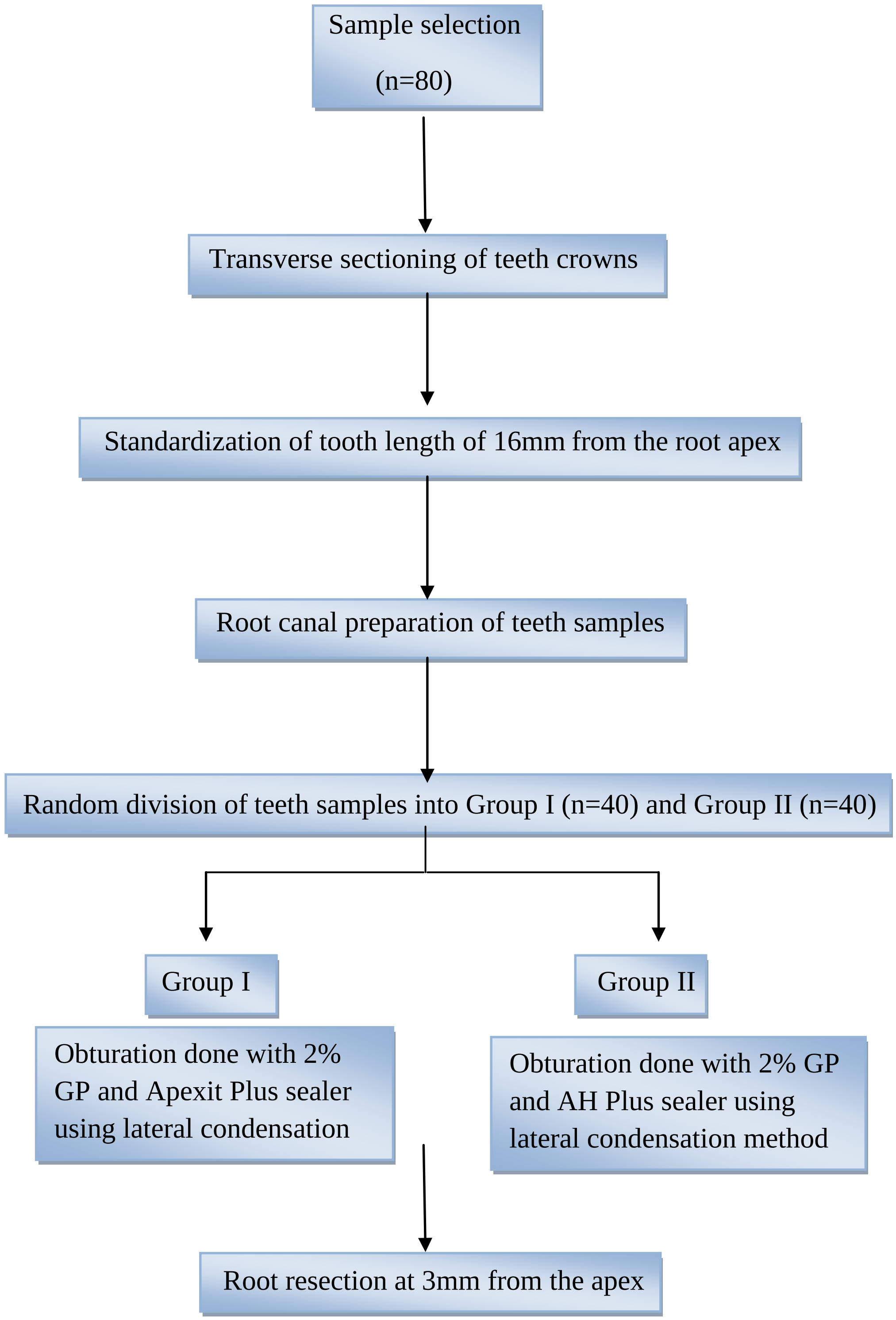
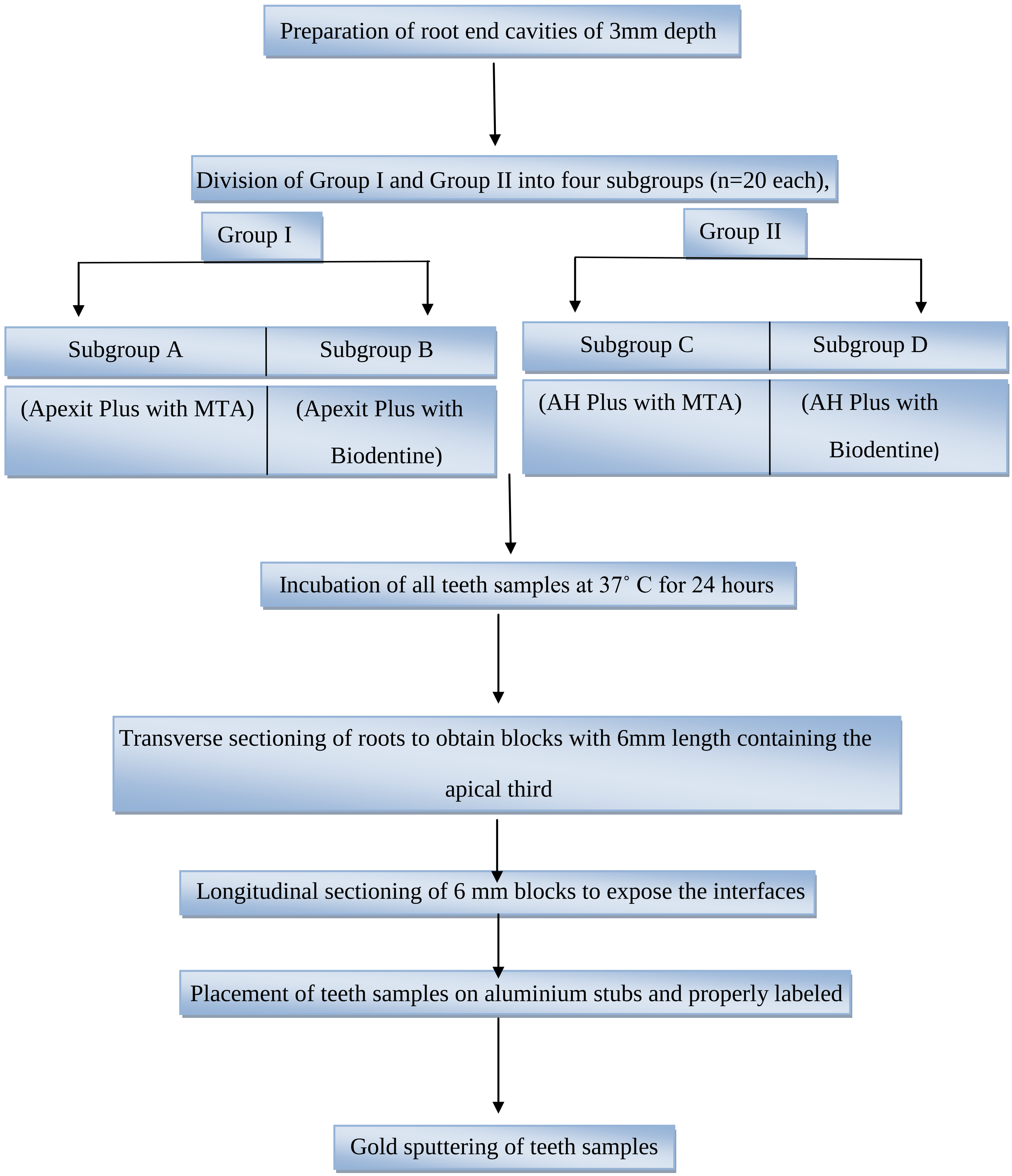

Sample selection
(n=80)
Transverse sectioning of teeth crowns
Standardization of tooth length of 16mm from the root apex
Root canal preparation of teeth samples
Random division of teeth samples into Group I (n=40) and Group II (n=40)
Group I
Group II
Obturation done with 2% GP and Apexit Plus sealer using lateral condensation
Obturation done with 2% GP and AH Plus sealer using lateral condensation method
Root resection at 3mm from the apex


Preparation of root end cavities of 3mm depth
Division of Group I and Group II into four subgroups (n=20 each),
Group I
Group II
Subgroup A
Subgroup B
Subgroup C
Subgroup D
(Apexit Plus with MTA)
(Apexit Plus with Biodentine)
(AH Plus with MTA)
(AH Plus with Biodentine)
Incubation of all teeth samples at 37˚ C for 24 hours
Transverse sectioning of roots to obtain blocks with 6mm length containing the apical third
Longitudinal sectioning of 6 mm blocks to expose the interfaces
Placement of teeth samples on aluminium stubs and properly labeled
Gold sputtering of teeth samples

Viewing of teeth samples under scanning electron microscope (SEM) at 600x

magnification to evaluate three different interfaces -

1. Root canal sealer – dentin interface
2. Root end filling material - dentin interface
3. Root canal sealer – root end filling material interface

SEM examination of the samples demonstrated variable gaps between the tested materials and the dentin walls. SEM analysis of each interface was evaluated separately as follows:

Interface 1: Between root canal sealer and dentin

a. Apexit Plus and dentin (n=40)

b. AH Plus and dentin (n=40)

Table 1. Comparison of mean values of gaps (μm) and standard deviation (SD) of interface between root canal sealer and dentin

Materials	Number of teeth	Mean ± SD (μm)
Apexit Plus	40	33.14 ± 21.29
AH Plus	40	36.35 ± 15.32

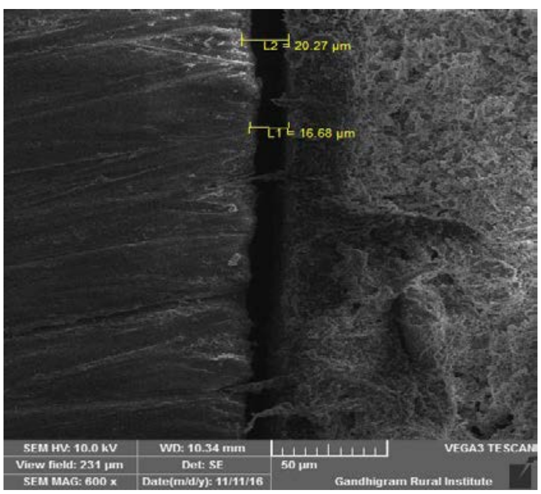


Fig 6.A Interface between Apexit Plus and dentin viewed under SEM at 600 x magnification.

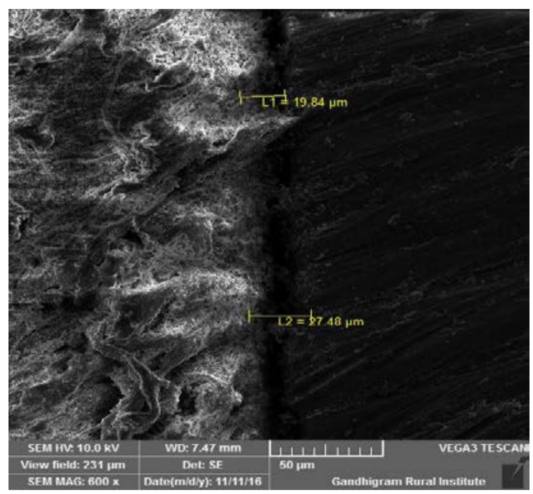
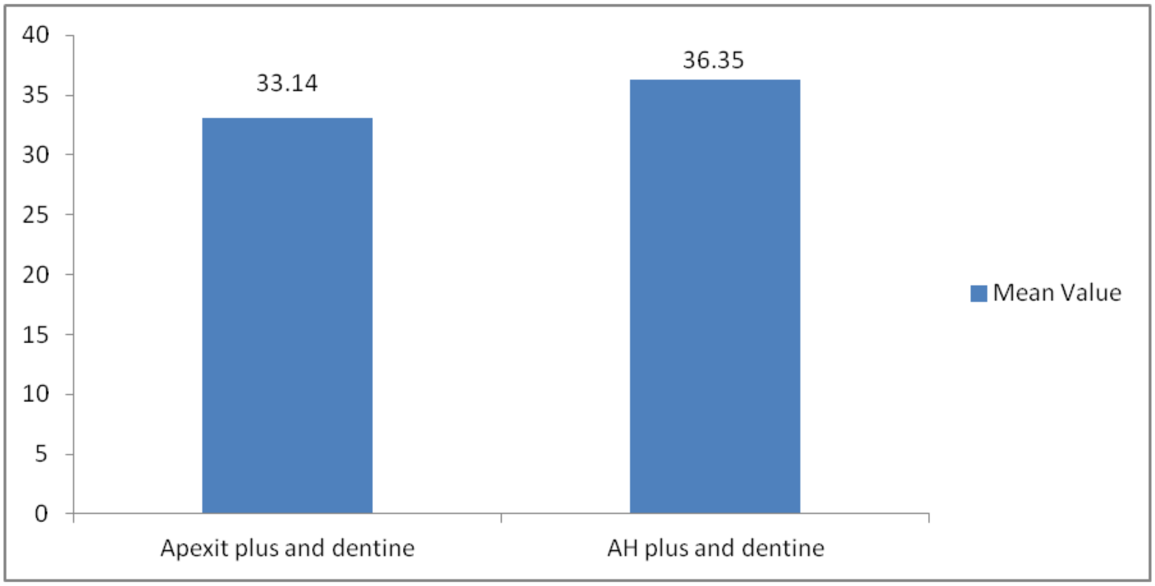


Fig 6.B Interface between AH Plus and dentin viewed under SEM at 600 x magnification.



Graph 1. Comparison of mean values of gaps between root canal sealers and dentin

The data did not follow normality. Hence, they were analyzed using Mann Whitney test and interpreted as there was no statistically significant difference between Apexit Plus and AH plus groups, since the p value is 0.133 ($p > 0.05$).

Interface 2: Between root end filling material and dentin

a. MTA and dentin (n=40)

b. Biodentine and dentin (n=40)

Table 2. Comparison of mean values of gaps (µm) and standard deviation (SD) of interface between root end filling material and dentin

Materials	Number of teeth	Mean ± SD (µm)
MTA	40	24.20 ± 17.52
Biodentine	40	20.17 ± 6.54

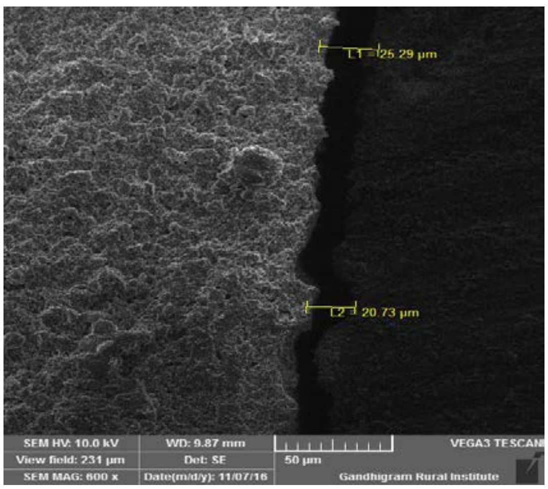


Fig 6.C Interface between MTA and dentin viewed under SEM at 600 x magnification.

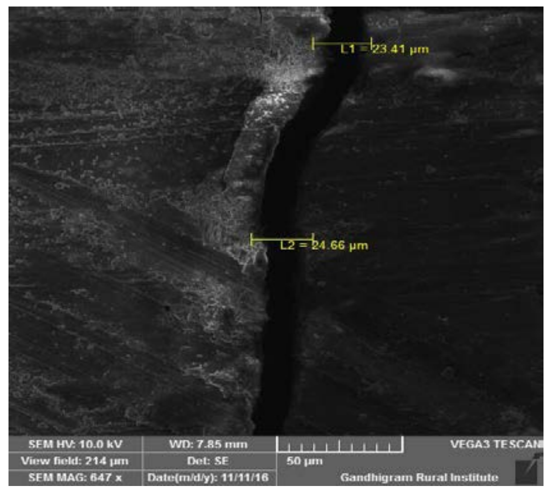
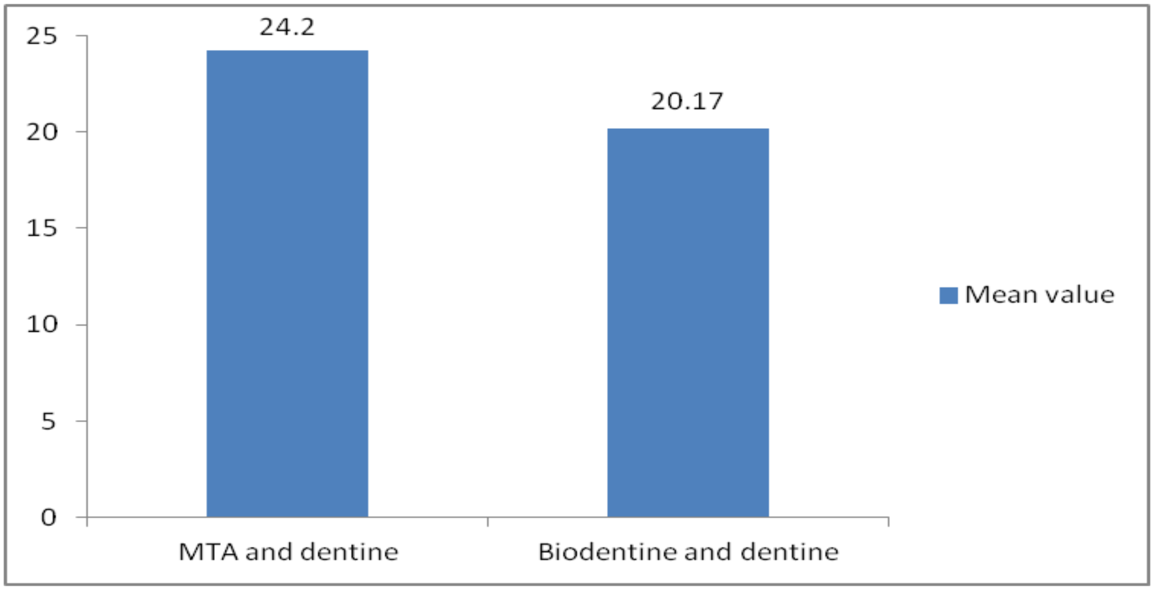


Fig 6.D Interface between Biodentine and dentin viewed under SEM at 600 x magnification.



Graph 2. Comparison of mean values of gaps between root end filling materials and dentin

The data did not follow normality. Hence, they were analyzed using Mann Whitney test and interpreted as there was no statistically significant difference between MTA and Biodentine groups, since the p value is 0.658 ($p > 0.05$).

Interface 3: Between GP with root canal sealer and root end filling material.

a. Apexit Plus and MTA (n=20)

b. Apexit Plus and Biodentine (n=20)

c. AH Plus and MTA (n=20)

d. AH Plus and Biodentine (n=20)

Table 3. Comparison of mean values of gaps (µm) and standard deviation (SD) of interface between root canal sealers and root end filling materials.

Subgroups	Materials	Number of teeth	Mean ± SD (µm)
A	Apexit Plus and MTA	20	16.82 ± 6.14
B	Apexit Plus and Biodentine	20	26.29 ± 18.17
C	AH Plus and MTA	20	18.55 ± 10.31
D	AH Plus and Biodentine	20	23.42 ± 13.77

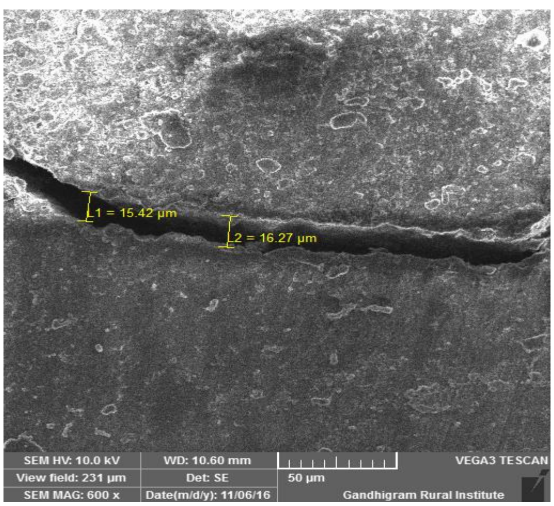


Fig 6.E Interface between Apexit Plus and MTA viewed under SEM at 600 x magnification

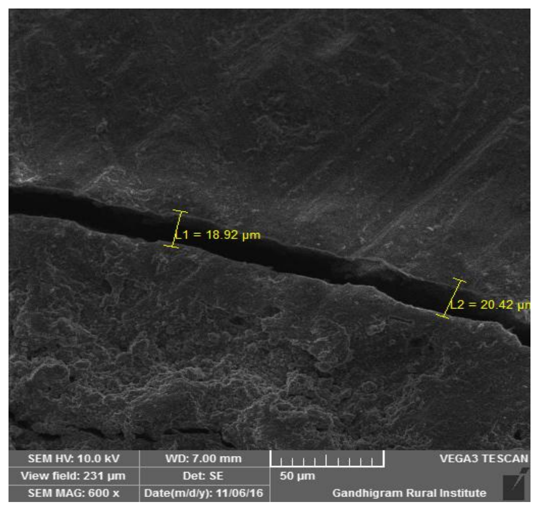


Fig 6.F Interface between Apexit Plus and Biodentine viewed under SEM at 600 x magnification

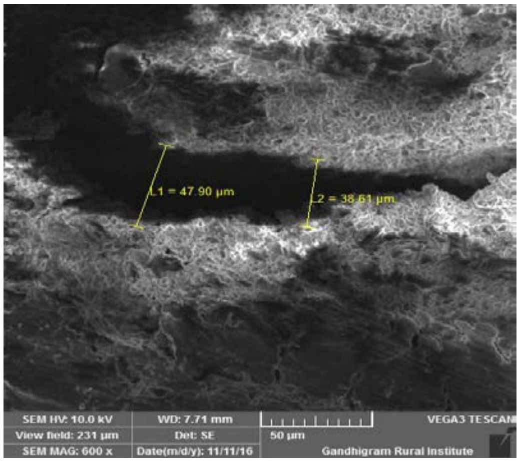


Fig 6.G Interface between AH Plus and MTA viewed under SEM at 600 x magnification

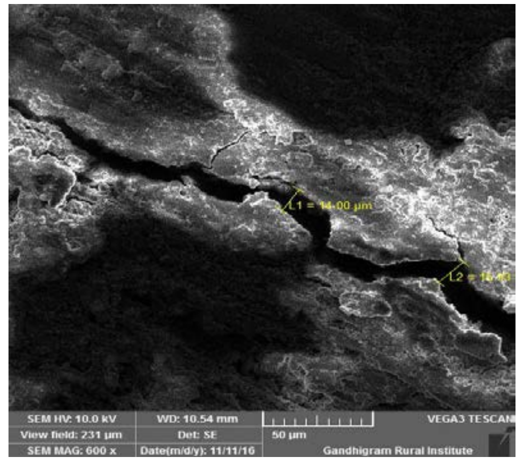
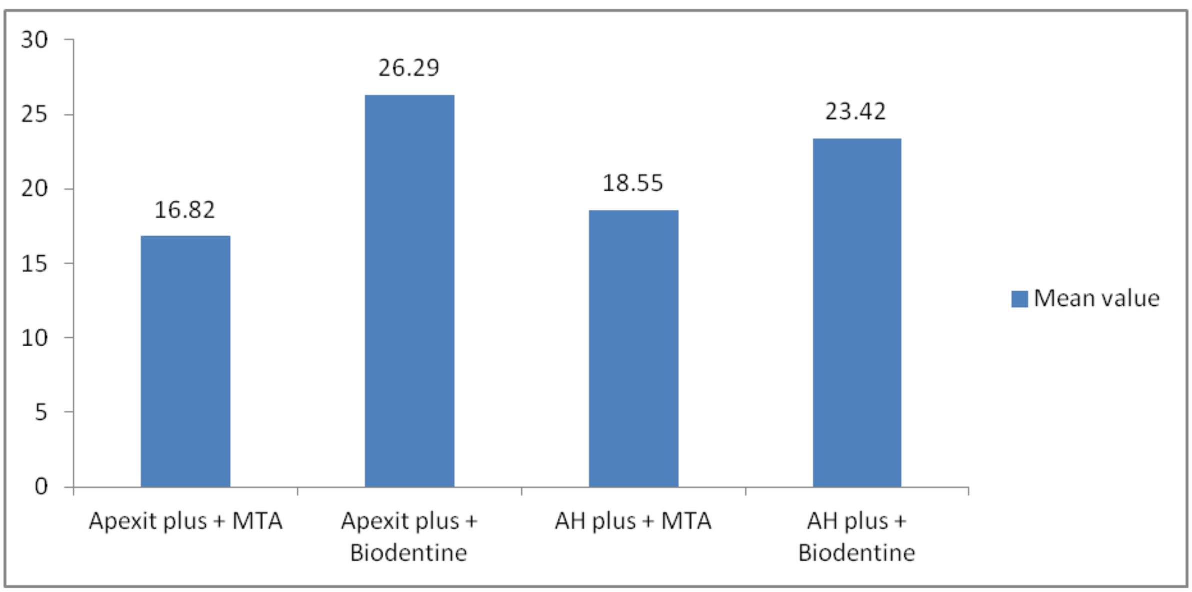


Fig 6.H Interface between AH Plus and Biodentine viewed under SEM at 600 x magnification



Graph 3. Comparison of mean values of gaps between root canal sealers and root end filling materials

All the four groups in the interface 3 did not follow normality. Hence, they were analyzed using Kruskal Wallis test and interpreted as there was a significant difference between all the four groups in the interface 3 with the p value of 0.026 ($p < 0.05$). Intergroup analysis was done using Mann Whitney test as they did not follow normality.

Mann Whitney test for Subgroup A (Apexit Plus with MTA) and Subgroup B (Apexit plus with Biodentine):

Statistical analysis revealed statistically significant difference between Apexit Plus with MTA and Apexit plus with Biodentine groups with p-value of 0.005 ($p < 0.05$). Based on mean gap values, Apexit Plus with MTA group presented smaller gaps when compared to Apexit plus with Biodentine group.

Mann Whitney test for Subgroup A (Apexit Plus with MTA) and Subgroup C (AH Plus with MTA):

Statistical analysis revealed no statistically significant difference between Apexit Plus with MTA and AH Plus with MTA groups, since the p-value is 0.968 ($p > 0.05$).

Mann Whitney test for Subgroup A (Apexit Plus with MTA) and Subgroup D (AH Plus with Biodentine):

Statistical analysis revealed no statistically significant difference between Apexit Plus with MTA and AH Plus with Biodentine groups, since the p-value is 0.213 ($p > 0.05$).

Mann Whitney test for Subgroup B (Apexit Plus with Biodentine) and Subgroup C (AH Plus with MTA):

Statistical analysis revealed statistically significant difference between Apexit Plus with Biodentine and AH Plus with MTA groups with p-value of 0.011 ($p < 0.05$). Based on mean gap values, AH Plus with MTA group presented smaller gaps when compared with Apexit Plus with Biodentine group.

Mann Whitney test for Subgroup B (Apexit Plus with Biodentine) and Subgroup D (AH Plus with Biodentine):

Statistical analysis revealed no statistically significant difference between Apexit Plus with Biodentine and AH Plus with Biodentine groups, since the p-value is 0.279 ($p > 0.05$).

Mann Whitney test for Subgroup C (AH Plus with MTA) and Subgroup D (AH Plus with Biodentine):

Statistical analysis revealed no statistically significant difference between AH Plus with MTA and AH Plus with Biodentine groups, since the p-value is 0.234 ($p > 0.05$)

Accomplishment of ideal root canal treatment is attributed to various essential factors such as proper instrumentation, biomechanical preparation and obturation. The main aim of this treatment is to remove the microbial entity and any future predilection of reinfection. In order to achieve this, proper seal is required to avoid any chance of proliferation of bacteria and future occurrence of any pathology. Sealer along with solid obturating material acts synergistically to create this hermetic seal[4,71].

Therefore, the primary objective of endodontic therapy is obtaining the hermetic seal of the apical foramen and total obturation of the root canal space[72.] The classic Washington study[2] had reported that 60% of endodontic failures was due to apical percolation. When nonsurgical root canal treatment fails to resolve periradicular lesions of endodontic origin or retreatment is contraindicated, periradicular surgery may be needed. This treatment consists of exposing the involved apex, resecting the root end, preparing root end cavity and filling this cavity with a root end filling material[73]. Investigators have reported that insufficient apical seal is the major cause of surgical endodontic failures[74,75], which leads to spread of microorganisms and their toxins beyond apex. Hence root-end cavities should be filled with biocompatible substances that prevent ingress of potential contaminants into the periradicular tissues[73].

The primary function of retrograde filling is to seal the root canal system when an adequate seal does not exist even after root resection. This prevents leakage of irritants from the root canal into the periradicular tissues, which could eventually cause treatment failure[61]. According to Mikko Altonen and Keijo Mattila[75], teeth with root end fillings had better healing rates than those that were not filled, provided that orthograde root filling were done. Joshua Lustmann et al.[76] also reported that a significantly higher success rate was found after retrofilling of roots with apparently well condensed obturation. These studies revealed that the root ends filled with retrograde filling materials resulted in greater healing success.

According to Gilheany et al[77], atleast 2 mm of root apex should be removed to minimize bacterial leakage from the canals. The study of the anatomical root apex shows that atleast 3mm of the root apex must be removed to reduce 98% of the apical ramifications and 93% of the lateral canals. A root end resection of less than 3mm do not most likely remove all of the lateral canals and apical ramifications which leads to risk of reinfection and eventual failure[78].

The plane of sectioning is an important consideration in technique of root resection. The root resection should be perpendicular to the long axis of the root devoid of bevels. A 45º buccolingual bevel is an option to facilitate the material insertion, however, it could increase the apical leakage because the permeability of the dentin tubules are more exposed by the bevel angle[36]. Bevelling also causes significant damage to the tissue structures such as buccal bone and misses the lingually positioned root apex, causing elongation of the canal and reduction of the root diameter, thereby weakening it[48]. The cutting bevel on the resected root end can be made perpendicular to the long axis of the root canal, thereby decreasing the number of exposed dentinal tubules and consequent apical leakage[79,80]. So, 90º angulations have been proved to be most acceptable method of root resection[45,81]. Hence, in this study the root ends were resected perpendicular to the long axis of the root.

Arens[82] suggested that after root resection, a Class I type cavity approximately 3 to 5 mm deep into root dentin should be prepared, with walls parallel to and coincident with the anatomic outline of the root canal space. The depth of penetration should be ideally 3mm[83]. Bradford R. Johnson[11] also suggested a root-end preparation depth of 3.0 mm.

Improvement in sonic and ultrasonic (US) retrotips have been of great benefit to root end treatment. US retrotips are advantageous over traditional apical surgery employed with high speed handpieces and burs as it creates smaller, better centered and better shaped root end cavities, thereby reducing the risk of perforations. Also, these devices can follow the long axis of the tooth and apical cavities can be prepared easily and safely[48].

The use of root canal sealers along with well adapted gutta-percha gives the operator a better chance to achieve a impervious seal[84]. Root canal ramifications, such as lateral, secondary and accessory canals can establish connection between the main root canal and periodontal ligament, as well as the apical foramen[85,86]. Several authors reported that localized periodontal problems might be associated with necrotic and infected root canal ramifications highlighting the importance of the capacity of the endodontic sealer to flow into these irregularities[85,87].

For many years, gutta-percha has been used as a core material with zinc oxide eugenol based sealers to obturate the root canal system in endodontic treatment. However, studies have shown that these sealers shrink upon setting and dissolve over a period of time and hence compromise the quality of the apical seal[24]. Recently, newer root canal sealers with antibacterial properties and adhesive properties have been developed. Apexit Plus, which is a calcium hydroxide based sealer and AH Plus which is a resin based sealer are shown to have better sealing abilities and long term stability than conventional sealers[41].

Marginal adaptation constitutes an indirect method to compare the sealing ability of root canal sealers and root end filling material[88]. Scanning Electron Microscope (SEM) has been used in this study to compare the marginal adaptation of root canal sealers and root end filling materials to the surrounding root dentin by using longitudinal sections of the tooth. These microscopes are known for their high magnification, good resolution, and larger depth of field which is not possible with other optical microscopes. The SEM uses electromagnets rather than lenses allowing the operator to have more control over the degree of magnification, thereby providing strikingly clear images[41].

Longitudinal sectioning was selected for the sample preparation in this study. However, conventional preparation of biologic samples before the SEM examination might be associated with the introduction of many artifacts. High vacuum evaporation can cause artifacts such as cracks in hard tissue samples, separation and lifting of the filling material from the surrounding tooth. In addition, there might be expansion or contraction of the tooth and/or filling material[61]. Hence, few previous studies evaluated the marginal adaptation of the filling materials to the canal wall using resin replicas to avoid artificial gaps within the interface[19]. Orosco et al.[37] reported that the samples can be directly viewed under SEM after gold sputtering for evaluation of marginal adaptation of the root end filling material and there is no need for creation of resin replicas, as direct SEM evaluation of the samples did not result in artificial gap formation. Hence, we used longitudinal sections of the tooth and examined the interface directly under SEM after performing gold sputtering[89,30].

Apical seal of root end filling materials is the single and most important factor in achieving success in surgical endodontics[67]. Various studies reported that MTA presented excellent apical sealing and demonstrated its superiority in comparison to other commonly used root end filling materials[90,28,29,91,92,93,94]. MTA due to its higher biocompatibility and sealing ability promotes better healing of the tissues when placed in contact with the dental pulp or periradicular tissues over the other available root-end filling materials, which has been proved by both *in vitro* and *in vivo* studies[95,96]. Its adaptation and properties are not affected by moisture as seen in various studies where it has been proved that there was no significant difference in its retention when a dry or wet cotton pellet was used during its packing into the cavity[97]. Moreover, apatite crystal formation has also been demonstrated along the MTA–dentin interface and within the interfacial dentin[98,99,100]. This apatite formation contributes to leakage reduction not only by filling the gap along the interface but also via dentine interactions such as intrafibrillar apatite deposition which is supported by the finding that immersion in PBS decreases marginal leakage of MTA apical plugs[101].

Biodentine is a newly introduced material with largely improved physical properties which can be used as a root end filling material. Biodentine’s improved physico-chemical properties like short setting time, high mechanical strength make it clinically easy to handle and compatible, not only with classical endodontic procedures, but also in restorative cases[45]. Biodentine shows apatite formation after immersion in phosphate solution**,** indicative of its bioactivity[102].

Hence, it is clear that, it is the sealing ability or the adaptability of a material which makes an ideal root end filling material or an ideal root canal sealer which prevents leakage of irritants and thereby aids in complete healing. So, this study was aimed at evaluating three different interfaces between root canal sealer and root end filling material which is of clinical relevance in choosing a root canal sealer or a root end filling material.

The three different interfaces (root canal sealers – dentin interface, root end filling materials – dentin interface, root canal sealer and root end filling material interface) were evaluated individually using scanning electron microscope (SEM).

Evaluation of interface between root canal sealer and dentin:

The evaluation of interface between root canal sealers (Apexit Plus, AH Plus) and dentin showed that the adaptation of Apexit Plus to root dentin was better compared to AH Plus with no statistically significant difference. The adaptation of Apexit Plus sealer to dentin was due to leaching of calcium and hydroxyl ions to surrounding tissues, thus filling the gaps[84]. The adhesion of AH Plus to root dentin was due to its better penetration into microirregularities because of its creep capacity and long setting time, which increases the mechanical interlocking between sealer and root dentin[85]. Cohesion among epoxy resin based sealer molecules further increases the resistance to removal and/or displacement from dentin[103].

The results of this study are in accordance with the study done by Salz et al[23], who stated that Apexit Plus had a better sealing ability when compared to AH Plus. The film thickness of a material describes its ability to adapt to the geometry of the canal wall. The lower the film thickness of the sealer, closer will be the adaptation of the material to the root canal. The film thickness of the Apexit Plus is 11µm compared to the film thickness of 28µm for AH Plus, which accounts for the better adaptation of Apexit Plus to the root dentin.

Evaluation of root end filing material and dentin:

The evaluation of interface between root end filling materials (MTA, Biodentine) and dentin showed that the adaptation of Biodentine to root dentin was better compared to MTA with no statistically significant difference.

MTA is a powder that consists of fine hydrophilic particles that sets in the presence of moisture[104]. The principal components are tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide[33]. During setting, calcium ions released from MTA reacts with tissue phosphates yielding hydroxyapatite, which is the basic matrix formed at MTA – dentin interface[100]. The sealing ability of MTA may be attributed to these physicochemical reactions.

Biodentine is similar to MTA in basic composition. The powder consists of tricalcium silicate, dicalcium silicate, calcium carbonate, and zirconium dioxide. The liquid consists of calcium chloride in aqueous solution with an admixture of polycarboxylate. After mixing, the calcium silicate particles of Biodentine, react with water to form a high pH solution containing $Ca2+$, $OH-$ and silicate ions. In the saturated layer, the calcium silicate hydrate (CSH) gel precipitates on the cement particles, whereas calcium hydroxide nucleates[14]. The CSH gel polymerizes over time to form a solid network and the release of calcium hydroxide increases the alkalinity of the surrounding medium. Saliva, as other body fluids, contains phosphate ions[105], an

interaction between the phosphate ions of the storage solution and the calcium silicate based cements leads to the formation of apatite deposits that can increase the sealing efficiency of Biodentine[106].

The results of this study is similar to the study done by Ravichandra P V et al[57]. According to the results of his study, Biodentine showed better marginal adaptation to root dentin compared to MTA. During the setting of Biodentine, an alkaline environment is achieved at the area of contact between Biodentine and dentin which opens the path through which the dentin substitute mass enters the exposed opening of the dentin canaliculi, thereby establishing a tight seal with dentin.

Han and Okiji et al[13] compared Biodentine and MTA with regard to calcium and silicon uptake by adjacent root canal dentin in the presence of phosphate buffered saline. The results showed that Biodentine had a better biomineralization ability than MTA, since Biodentine specimens showed wider calcium and silicon rich dentin areas and larger incorporation depths than MTA. Biodentine released larger amounts of calcium and consequently produced larger amounts of calcium phosphate precipitates, thereby providing a better adaptation to root dentin.

Evaluation of interface between root canal sealer and root end filling material:

Comparing Subgroups A, B, C and D, the adaptation of Subgroup A was significantly higher followed by subgroups C, B and D.

According to the results of this study, MTA showed better adaptation to both Apexit Plus and AH Plus compared to Biodentine. MTA is hydrophilic and it requires moisture to set. MTA shows absorption of dihydrogen monoxide during hydration of powder leading to expansion during setting which may be the reason for its excellent sealing ability[107]. According to Rosa et al[27], the great adaptation of the MTA to the obturating material and root canal sealer may be attributed to its physical and chemical properties.

Thus, the material expands during setting, which must have played a role in its superior adaptation to the root canal filling[108,109].

MTA releases calcium and hydroxyl ions which interacts with a phosphate containing synthetic body fluid resulting in the formation of apatite like interfacial deposits. These deposits fill up the gaps and improve the frictional resistance of MTA to root canal walls. The sealing ability of MTA is attributed to the formation of this non bonding, gap-filling apatite deposits[110].

Adhesion or adaptation of root end filling material with root dentin and with root canal sealer is of prime importance, as both the materials must be able to remain adapted to each other to maintain adequate apical seal, when the teeth are subjected to mechanical forces during function or operative and surgical procedures[111].

Sealers also should demonstrate good adhesive properties and better adaptation not only to root dentin, but also with the root end filling material for adequate apical seal, which leads to greater strength of the restored tooth and provide greater resistance to tooth fracture, thus decreasing the chances of endodontic failure and increasing the longevity of an endodontically treated tooth[112].

The paramount importance for successful endodontic treatment is thorough cleaning, shaping and sealing of entire root canal system with an inert material which should be highly compatible with the periapical tissues and provide better marginal adaptation to the canal walls. So, we designed a study methodology by using root end filling materials like MTA, Biodentine and root canal sealers like Apexit Plus, AH Plus to evaluate three different interfaces between root canal sealers (Apexit Plus, AH Plus), root end filling materials (MTA, Biodentine) and dentin using scanning electron microscope (SEM).

Henceforth, we have done this in vitro study by selecting eighty extracted maxillary incisors with mature apices and free of caries. They were decoronated to create a standardized tooth length of 16 mm from the root apex. After determining the working length, the root canals were instrumented up to size 40 K file using step back technique. The canals were irrigated with 5% sodium hypochlorite followed by saline rinse between every instrument change. The final irrigation was done using 17% EDTA followed by saline and were then dried with paper points.

The teeth were randomly divided into two groups of forty samples each as, Group I: obturation done with 2% GP and Apexit Plus sealer using lateral condensation method and Group II: obturation done with 2% GP and AH Plus sealer using lateral condensation method.

Roots were resected perpendicular to the long axis of the root using a diamond disc at 3 mm from the apex and retrograde cavities were prepared using ultrasonic retrotip to a depth of 3 mm.

The two groups, Group I and Group II were further subdivided into four subgroups of twenty samples each as, Subgroup A: Apexit Plus with MTA, Subgroup B: Apexit Plus with Biodentine, Subgroup C: AH Plus with MTA and Subgroup D: AH Plus with Biodentine.

Both the root end filling materials were mixed according to manufacturer's instructions and the retrograde cavities were filled according to the subgroups divided. All the teeth were wrapped in a wet gauze and placed in an incubator at 37˚C for 24 hours for the root end filling materials to set completely. The roots were transversally sectioned with diamond disc to obtain blocks of 6mm length containing the apical third. The blocks were again longitudinally sectioned to expose the interface between the sealer and root end filling material. The samples were placed on metal stubs, gold sputtered and viewed under SEM at 600 x magnification and the widest gap values of the interfaces were recorded.

Data were analyzed using Mann Whitney test for root canal sealer – dentin interface and root end filling material – dentin interfaces. Interface between root canal sealer and root end filling material were analyzed using Kruskal Wallis test followed by Mann Whitney test with $p < 0.05$ level of significance.

Based on the results, the mean gap values of interface between Apexit Plus and AH Plus with dentin were 33.14µm and 36.35µm respectively which were not statistically significant. The mean gap values of interface between MTA and Biodentine with dentin were 24.20µm and 20.17µm respectively which were not statistically significant. The mean gap values of interface between Apexit Plus with MTA, Apexit Plus with Biodentine, AH Plus with MTA and AH Plus with Biodentine were 16.82µm, 26.29µm, 18.55µm and 23.42µm respectively.

Among the subgroups, Subgroup A (Apexit Plus with MTA) and Subgroup B (Apexit Plus with Biodentine) showed statistically significant difference with Subgroup A presenting smaller gaps, when compared to Subgroup B. Subgroup B (Apexit plus with Biodentine) and Subgroup C (AH Plus with MTA) showed statistically significant difference with Subgroup C presenting smaller gaps. The other subgroups showed no statistical significant difference based on mean gap values.

Though the study has evaluated different interfaces between root canal sealer and root end filling material, it was of short term. Further studies on evaluation of adaptation of the materials over a long duration is very essential. As time has an influence on adaptation of various materials, long term studies which predict not only the immediate effect of the setting reaction of the materials but also the capability of the materials to maintain the apical seal created is mandatory to reveal the success of a material as root canal sealer or root end material.

Within the limitations of the present study, it can be concluded that

1. Both the sealers (Apexit Plus and AH Plus) can be used as potent root canal sealers.

2. Both the root end filling materials (MTA and Biodentine) are considered to have better marginal adaptation with dentin.

3. MTA has got better adaptation to both AH Plus and Apexit Plus sealers compared to Biodentine.

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ANNEXURE -1

Ref:UT:BDSC:IRB-EC/2014 Date:18.11.2014

From

Institutional Review Board-Ethical committee,
Best dental science college,
Madurai .

To

The Controller of Examinations ,
The Tamil Nadu DR.MGR Medical University,
No. 69, Anna salai,
Guindy,
Chennai-600 032

Sir/Madam

The Dissertation topic titled " SEM STUDY OF THREE DIFFERENT INTERFACES BETWEEN SEALER AND ROOT END FILLING MATERIALS – AN IN VITRO STUDY" submitted by DR.C.A.ANAND YOKESH postgraduate student has been approved by Institutional Review Board of Best Dental Science College on 18.11.2014.

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INSTITUITONAL REVIEW BOARD-ETHICAL COMMITTEE
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